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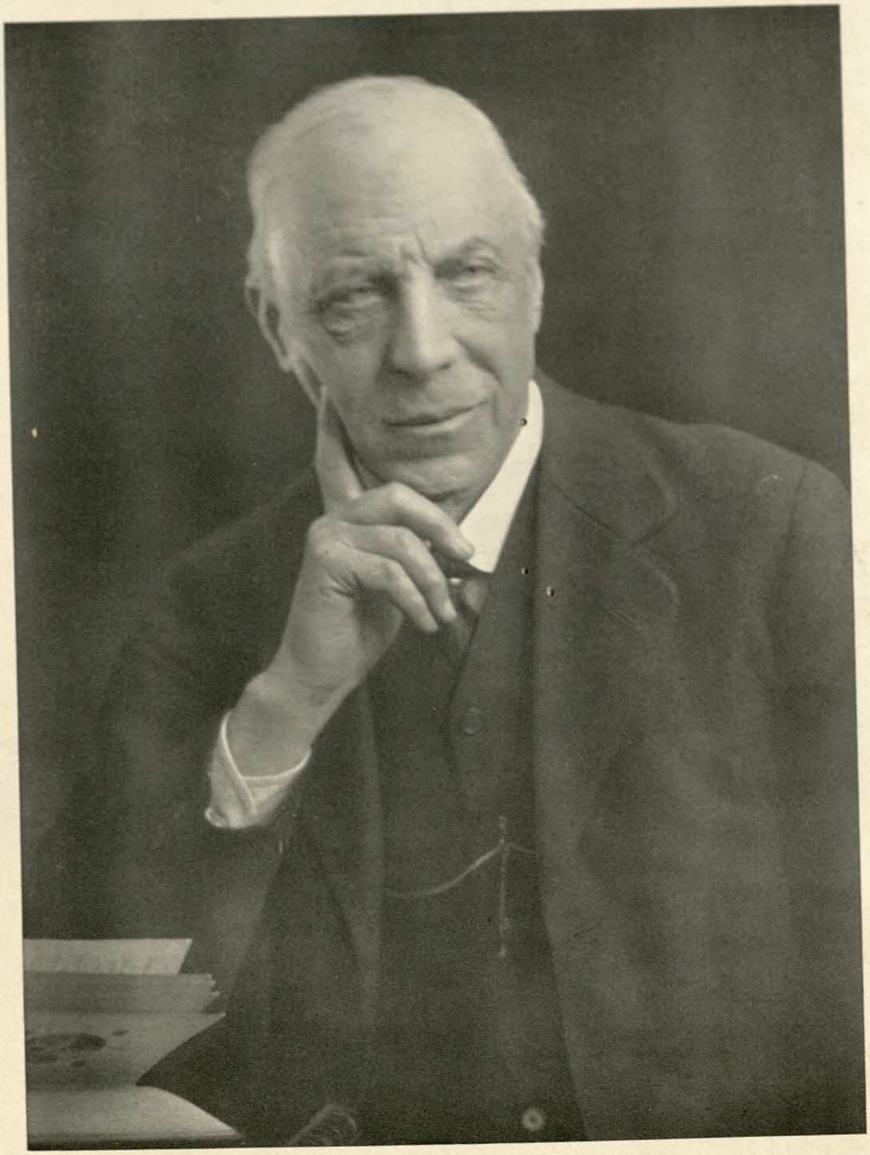

Sir EDWARD SHARPEY-SCHAFER

QUARTERLY JOURNAL
OF EXPERIMENTAL PHYSIOLOGY
AND COGNATE MEDICAL SCIENCES

1908–1958

SIR EDWARD SHARPEY-SCHAFER founded this journal in January 1908 and thus we can now celebrate our fiftieth birthday. Previously the *Journal of Physiology*, founded in 1878 by Sir Michael Foster, Professor of Physiology in Cambridge, was the only medium for the publication of physiological papers in Britain. J. N. Langley, who succeeded Foster as both proprietor and editor, was a great editor, but he was very punctilious and an autocrat. Although he frequently improved the papers he edited there were many physiologists who welcomed the appearance of an alternative journal.

The opportunity of starting a new journal came when I. G. McKendrick retired from the Chair of Physiology at Glasgow and from the editorship of the *Journal of Anatomy and Physiology*. Schafer was approached to become editor of the physiological part. He agreed on condition that the physiological section was completely separated from the anatomical section. However, only four of the subscribers to the old journal were willing to subscribe to a new physiological section, and the scheme was dropped. Sherrington, who was consulted by Schafer, advised strongly that the new journal should be independent and without any ties with anatomy. The establishment of a new journal under the title of the *Quarterly Journal of Physiology* was advertised, with the support of Sherrington, Starling, Gotch, Halliburton and Waller. This advertisement drew vigorous protests by letter and telegram from Langley who feared that the new journal would be confused with the *Journal of Physiology*. The title was therefore altered to the *Quarterly Journal of Experimental Physiology* and, by letter and circular, the promoters of the new journal tried to make it clear that they were in no way activated by hostility to Langley or to the *Journal of Physiology*.

However, both journals flourished, for physiology was rapidly expanding at the time. The *Quarterly Journal* was given by Schafer on his retirement in 1932 to Professor I. de Burgh Daly, who arranged for it to become the property of three trustees, the Professors of Physiology, Pharmacology and Anatomy at Edinburgh.

When the journal was founded it was hoped that papers in allied fields particularly in histology would be attracted to the journal. This policy has been maintained and papers on histological topics which bear on physiological problems are still welcomed and produced with the high standard of block making insisted upon by Schafer. The words "and Cognate Medical Sciences" were added to the title in 1938 during the editorship of Professor I. de Burgh Daly. Papers on experimental zoology and pathology are still very welcome.

The tradition that authors are allowed a little more freedom than is customary in the *Journal of Physiology* is, we hope, still maintained.

REVIEW ARTICLE

INTRINSIC MECHANISMS OF THE LUNG.* By I. DE BURGH DALY.

From the Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge.

(*Received for publication 7th June* 1957)

The case for an active control of the lesser circulation by nervous, humoral or gaseous stimuli rests upon the claim that pulmonary vasomotor responses can be obtained under experimental conditions which wholly eliminate the participation of passive effects. Passive regulatory mechanisms of the lesser circulation are discussed and the experimental criteria regarded as establishing the elimination of passive cardiomotor, respiratory, bronchomotor and bronchial vascular effects described. With these criteria fulfilled it has been shown that in the dog the upper thoracic sympathetic outflow contains adrenergic vasoconstrictor fibres to the pulmonary vascular bed proper which when stimulated may increase the pulmonary vascular resistance by sixty per cent or more.

The upper thoracic sympathetic outflow and the vagi of the dog contain fibres which on stimulation may lower the pulmonary vascular resistance by direct action or as a result of bronchial vascular effects. The sympathetic fibres concerned are atropine resistant, the vagal fibres atropine sensitive.

The evidence for an active pulmonary vasomotor control in man is examined and suggestions put forward for future investigations. The contribution which studies in comparative physiology and comparative pathology can make in furthering our knowledge of pulmonary vascular function in health and disease is discussed.

IT is well recognized that passive cardio-respiratory events predominate in the regulation of the lesser circulation, but opinions differ as to whether nervous, humoral and gaseous stimuli exert an active control on the pulmonary vascular bed proper. Criticisms have been levelled against the conception of an active control on *a priori* grounds in that it is not essential [Cournand,

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* This article is an account of the experimental work and ideas which formed the basis of the Bertram Louis Abrahams Lecture delivered at the Royal College of Physicians of London on 5th July 1956.

1947], or that the experimental results interpreted as evidence of pulmonary vasomotor activity are due to passive cardiac, respiratory and bronchomotor effects [Hess, 1930; Wagner, 1935; Rodbard, 1953] or represent a vestigial function [Dixon and Hoyle, 1928; Hamilton, Woodbury and Vogt, 1939].

The significance of teleological speculations can only be assessed in the light of knowledge gained by experiment and therefore it is important first to determine whether pulmonary vasomotor responses can be obtained under experimental conditions which wholly eliminate the participation of passive effects, for it is on this claim that the case for an active control rests. For this purpose an appreciation of the nature of passive mechanisms is required and of the steps which have to be taken for excluding them.

PASSIVE REGULATORY MECHANISMS OF THE LESSER CIRCULATION AND EXPERIMENTAL METHODS USED FOR THEIR ELIMINATION

Changes in cardiac output produce corresponding alterations in pulmonary blood flow and the only certain method of excluding passive cardiac effects on the lung vessels is to use controlled perfusion of the lungs. In experiments in which perfusion is confined to the pulmonary vascular bed it is carried out at a constant head of inflow pressure or at a constant volume inflow and the effect of the stimulus measured by recording changes in lung blood flow or in pulmonary arterial pressure respectively. Free outflow from a cannula inserted in the left auricle prevents appreciable changes in left auricle pressure during tests [Daly, Elsden, Hebb, Ludány and Petrovskaia, 1942]. Simple perfusion in this manner will detect pulmonary vascular responses to inhalation of gas mixtures and to intravascular drug injections but not to nerve stimulation because—at any rate in the dog—the lung nerves, ganglia and possibly some of the smooth muscle require for survival oxygenated blood from the bronchial circulation. The failure to recognize this requirement has been largely responsible for the view that pulmonary vasomotor nerve function is absent or at the best vestigial in character. In order to maintain the viability of these tissues additional controlled perfusion of the bronchial circulation is carried out in lungs isolated from the body [Daly and Euler, 1932; Daly, 1956 *a*] or in the perfused living animal preparation, in which the greater and lesser circulations are perfused by two separate blood pumps [Daly *et al.*, 1942].

Unfortunately, bronchial vascular system perfusion may introduce another source of passive effect on the pulmonary vessels because there are communications through the bronchial vascular system between the greater and lesser circulations. Through these the effects of a change in systemic arterial pressure [Berry and Daly, 1931] or of resistance of the bronchial arteries due to bronchial vasomotor activity [Daly and Euler, 1932; Bruner and Schmidt, 1947] may be transmitted to the pulmonary vascular bed [Daly and Hebb, 1942]. In certain lung diseases the bronchial vessels and their communications with the lesser circulation enlarge and, in these circumstances, changes in systemic arterial pressure or in resistance of the bronchial circulatory system will become of increasing importance in determining the pulmonary vascular resistance as measured by conventional methods. In the laboratory the only certain method for excluding such passive bronchial vascular effects is to reduce the systemic arterial pressure to zero whilst making tests for active responses of the pulmonary vessels [Daly, 1956 *b*].

Passive respiratory effects due to respiratory centre activity in isolated perfused lungs naturally do not present a problem. In the perfused living animal any possibility of respiratory efforts affecting the lungs can be prevented by practising controlled lung ventilation with a widely opened thorax. Muscle paralysants such as decamethonium can also be used for this purpose. Even under these conditions as well as in excised perfused lungs stimulation of certain pulmonary nerves, the inhalation of various gases and the administration of drugs sometimes cause active responses of the respiratory tree—generally termed bronchomotor—which passively affect the lung blood vessels. There are several reasons for this.

In lungs rhythmically ventilated by a pump delivering a constant volume of air at each stroke, bronchoconstriction leads to an inspiratory increase in tracheal pressure and to retention of air in alveoli due to the elastic recoil of the lungs being temporarily insufficient to force all the air back through the constricted bronchioles during expiration. Air may also be squeezed into and trapped within the alveoli by bronchiolar constriction [Beer, 1892; Rodbard, 1953]. The resulting increase in intra-alveolar pressure compresses the capillaries and increases pulmonary vascular resistance [de Jager, 1885; Dale and Narayana, 1935]. On the other hand, when bronchoconstriction takes place in lungs ventilated at a constant peak pressure, although the intratracheal pressure does not change, the intra-alveolar pressure and therefore that exerted on the capillaries may rise or fall depending upon the phase of the respiratory cycle, during which the bronchiolar obstruction occurs [Daly and Hebb, 1942].

These are considerations which show that a pulmonary vascular response cannot be regarded as unequivocal evidence of an active control unless it is demonstrably unaccompanied by a bronchomotor response. There are other reasons for insisting upon this criterion. The lung blood vessels may also be passively influenced by reactions of the respiratory tree of which we are less well informed, such as contraction of the interstitial muscles postulated by Baltisberger [1921], secretion of the bronchial glands and possibly changes in surface tension of the respiratory tree lining [Neergaard, 1929; Pattle, 1955]. As far as we know, every kind of bronchomotor activity causes changes in lung viscance and/or elastance; these can be measured separately or collectively as alterations in total lung hindrance [Bayliss and Robertson, 1939; Mount, 1956]. In our own investigations lung hindrance changes were regarded as absent during tests for pulmonary vasomotor nerve activity if the tidal air remained constant when either positive or negative pressure ventilation was used by the methods of Konzett and Rössler [1940] and Daly *et al.* [1942] respectively.

These methods detect changes in lung viscance and elastance but do not distinguish between them; they do not detect synchronous changes in viscance and elastance of similar magnitude and of opposite sign, but this double event appears to be so unlikely that it can be disregarded. It might be argued that in lungs in which some of the bronchioles were already constricted or obstructed a superimposed bronchomotor response of the unventilated regions beyond them would not be detectable by tidal air measurements, and so any resulting passive effects on the lung blood vessels in the unventilated area would be mistakenly interpreted as an active vascular response. There is, however, no reason to suppose that bronchomotor responses to pulmonary nerve stimulation or to the injection of drugs are confined to unventilated lung areas, and therefore it is presumed that the tidal air record would reveal the accompanying bronchomotor changes in the ventilated areas. Also it would be expected that if bronchomotor responses occurred in unventilated regions they would cause detectable passive hindrance changes in adjacent ventilated regions, the hindrance of which is recorded.

From these arguments it might be thought that in unventilated lungs a bronchomotor response would have no passive effects on the pulmonary vascular bed. There is, however, the possibility that air may be squeezed into or released from the alveoli and in this way may cause changes in intra-alveolar pressure. Another point for consideration is whether contraction of the bronchial muscles compresses vessels of the pulmonary vascular bed proper. The histological evidence militates against this although bronchial vessels might be compressed because they pass through the bronchial muscle mesh [Miller, 1937].

The intimate anatomical relations between the respiratory tree, the pulmonary vascular bed and the bronchial vascular system are responsible for the difficulty both of separating their responses from one another and of so directing a stimulus that it acts exclusively on the desired tissue element. The air-ways form a common route for the application of gaseous stimuli to the lining of the whole of the respiratory tree as well as to the more peripheral parts of the pulmonary vascular bed. On the other hand, smooth muscle stimulants injected into the lesser circulation act first on all parts of the pulmonary vascular tree, then on the more peripheral portions of the air-ways, and after recirculation pass into the bronchial vascular system to affect the more proximal portions of the respiratory tract. In this event the vascular and bronchomotor responses take place in two stages separated by an interval which depends upon the recirculation time. The picture may be further complicated if the injected substances passing through the bronchial circulation modify the activity of nerve ganglia, the postganglionic fibres of which supply the bronchial muscles and pulmonary vascular bed. These considerations underline further the necessity for planning experiments in which the blood flow through the bronchial vascular system can be controlled at will. Since many smooth muscle stimulants, whether inhaled or injected into the blood stream, affect both the blood vessels and the air-ways the successful demonstration of a specific action on the vessels—depending as it does on the elimination of the bronchomotor response—can only be achieved if the threshold responses of the vessels and bronchial muscles differ widely.

PULMONARY VASOMOTOR NERVE FIBRES

There are particular difficulties in the dog in demonstrating the activity of these fibres because those in the sympathetic chain are intermingled with cardio-accelerator, cardio-augmentor and bronchodilator fibres, whereas those in the cervical vagi are associated with cardio-inhibitory and bronchoconstrictor fibres. The thoracic vagosympathetic nerves contain all five types of fibres. Fortunately, the vascular response is not always accompanied by changes in lung hindrance, possibly because the nerve stimulated does not contain bronchomotor fibres, or, if they are present, because their stimulation threshold is far higher than that of the vasomotor fibres. In other circumstances when the vasomotor and bronchomotor responses accompany one another and cannot be separated by varying the electrical parameters of the nerve stimulus it is frequently possible to suppress the bronchomotor effect by drugs without affecting the vascular response.

I now propose to furnish evidence obtained from lung perfusion preparations to show that the pulmonary vascular resistance is controlled by both sympathetic and vagal fibres under conditions which eliminate passive cardiac, respiratory and bronchomotor effects. Culminating evidence that sympathetic nerve stimulation which gives rise to an increase in pulmonary vascular resistance is an effect on the pulmonary vessels proper and is not secondary to bronchial vascular effects will also be presented.

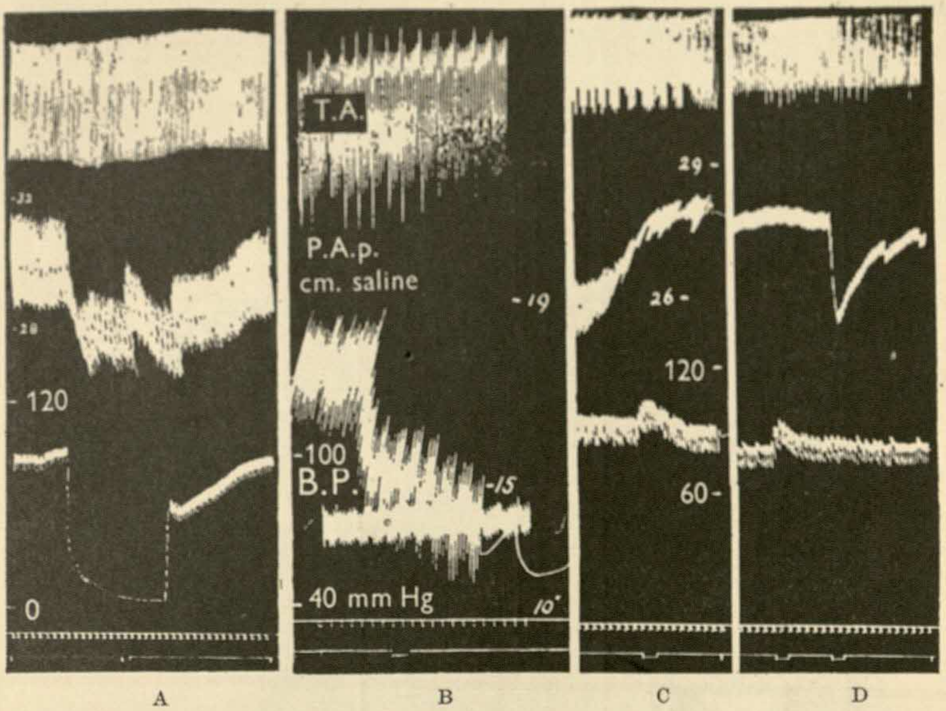
Upper Thoracic Sympathetic Outflow.—The predominant effect of electrical stimulation of the upper thoracic sympathetic chain as far caudal as T5 is an increase in pulmonary vascular resistance which occurs in the absence of changes

TABLE I.—PULMONARY VASCULAR RESISTANCE (P.V.R.) RESPONSES (UNACCOMPANIED BY CHANGES IN LUNG HINDRANCE) TO STIMULATION OF PULMONARY NERVE PATHWAYS IN PERFUSED LIVING ANIMAL (DOG) PREPARATIONS UNDER CONTROLLED CONDITIONS OF PERFUSION AND LUNG VENTILATION.

Method of lung perfusion	Method of lung ventilation	Stimulation of	P.V.R. response	
C.V.I.	N.P.V.	Upper thoracic sympathetic chains	+ rarely −	Daly, Elsden, Hebb, Ludány and Petrovskaia, 1942
		Stellate ganglia	+	
		Middle cervical ganglia	+*	Daly, Duke, Hebb and Weatherall, 1949
		Thoracic vagosympathetic nerves	+*	Daly and Hebb, 1952, 1954
		Right cervical vagosympathetic nerve	−	
		Left cervical vagosympathetic nerve	−	
		Left cervical vagosympathetic nerve	+*	
C.P.I.	P.P.V.	Upper thoracic sympathetic chains	+	Daly, Duke, Linzell and Weatherall, 1952 Daly, Linzell, Mount and Waites, 1954

C.P.I. = constant pressure inflow. C.V.I. = constant volume inflow.
N.P.V. = negative pressure ventilation. P.P.V. = positive pressure ventilation.
* = atropinized preparation.

in lung hindrance in isolated perfused lungs and in perfused living animal preparations irrespective of whether positive or negative pressure ventilation is used, or whether perfusion is carried out at a constant volume or constant pressure blood inflow (Table I and fig. 1C). The cell stations for the fibres concerned have been shown by ganglionic blocking agents—nicotine applied locally to the ganglia or hexamethonium given intravenously—to reside in the stellate and middle cervical ganglia [Daly *et al.*, 1949]. The post-ganglionic fibres reach the lungs chiefly by way of the thoracic vagosympathetic nerves so that when testing the effects of stimulating these nerves atropine has generally to be added to the perfusate to prevent vagal bronchomotor effects. Some of the pre- and post-ganglionic fibres from the thoracic sympathetic outflow loop or give rise to axon collaterals in the cervical vagosympathetic nerves (fig. 2, 1–4) [Daly and Hebb, 1942]. There is suggestive but not conclusive evidence that both the superior cervical and the nodose ganglia contain cells which project post-ganglionic pulmonary vasomotor fibres (fig. 2, 5).



A B C D

FIG. 1.—Perfused living animal preparations (dog). Perfusion of pulmonary circulation at constant blood volume inflow in each case. Effect of stimulating.

A, right middle cervical ganglion after atropine [Daly *et al.*, 1949].
No perfusion of bronchial circulation during stimulation.
B, right cervical vagosympathetic nerve [Daly and Hebb, 1952].
C, right thoracic sympathetic chain [Daly *et al.*, 1949].
D, right then left thoracic sympathetic chain [Daly *et al.*, 1949].
B, C and D were not atropinized preparations.

B.P. = systemic arterial pressure; P.A.p. = pulmonary arterial pressure; T.A. = tidal air.

The pulmonary vascular resistance increase which stimulation of this sympathetic path produces is enhanced by cocaine and reversed by ergotoxine in fully atropinized perfusion preparations and so suggests that the post-ganglionic vasoconstrictor fibres are adrenergic in character. The vasodilator fibres unmasked by ergotoxine may also be adrenergic but other possibilities cannot be excluded [Daly *et al.*, 1949]. Modifications of the pulmonary vascular response by these diagnostic agents is further evidence that the response is not secondary to vagal bronchomotor effects.

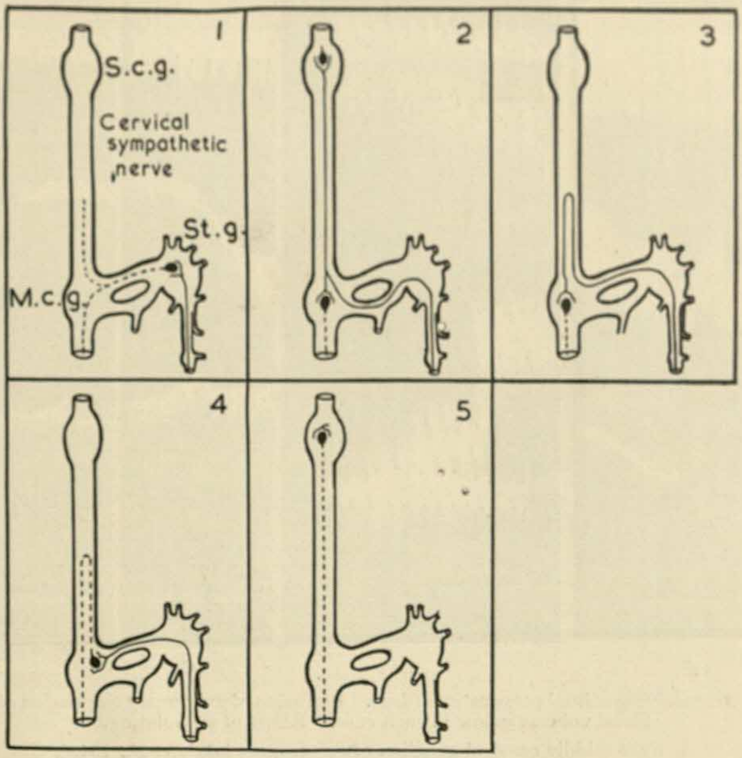


FIG. 2.—Probable pathways of pulmonary vasomotor nerve fibres in the dog. S.c.g. = superior cervical ganglion; m.c.g. = middle cervical ganglion; St.g. = stellate ganglion.

Before it can be concluded that these fibres exert an active control on the pulmonary vascular bed proper it must be shown that the responses to their stimulation are not due to passive bronchial vascular system effects. In experiments on *atropinized* perfused lungs stimulation of the sympathetic pulmonary nerve path has raised the pulmonary vascular resistance sixty per cent or more in the absence of a functional bronchial vascular system and of a change in lung hindrance [Daly, 1956 *a*]. Examples of such responses are shown in figs. 3 and 4. It is therefore difficult to escape the conclusion that, in the dog, the upper thoracic sympathetic outflow contains constrictor fibres to some portion of the pulmonary vascular bed proper. We shall see, however, that this same nerve pathway also carries pulmonary vasodilator nerve fibres.

Pulmonary Vasodilator Fibres.—All the fibres which I have mentioned would increase the resistance to blood flow through the lungs. No one has been able to see much use for such fibres—a point which I will return to later.

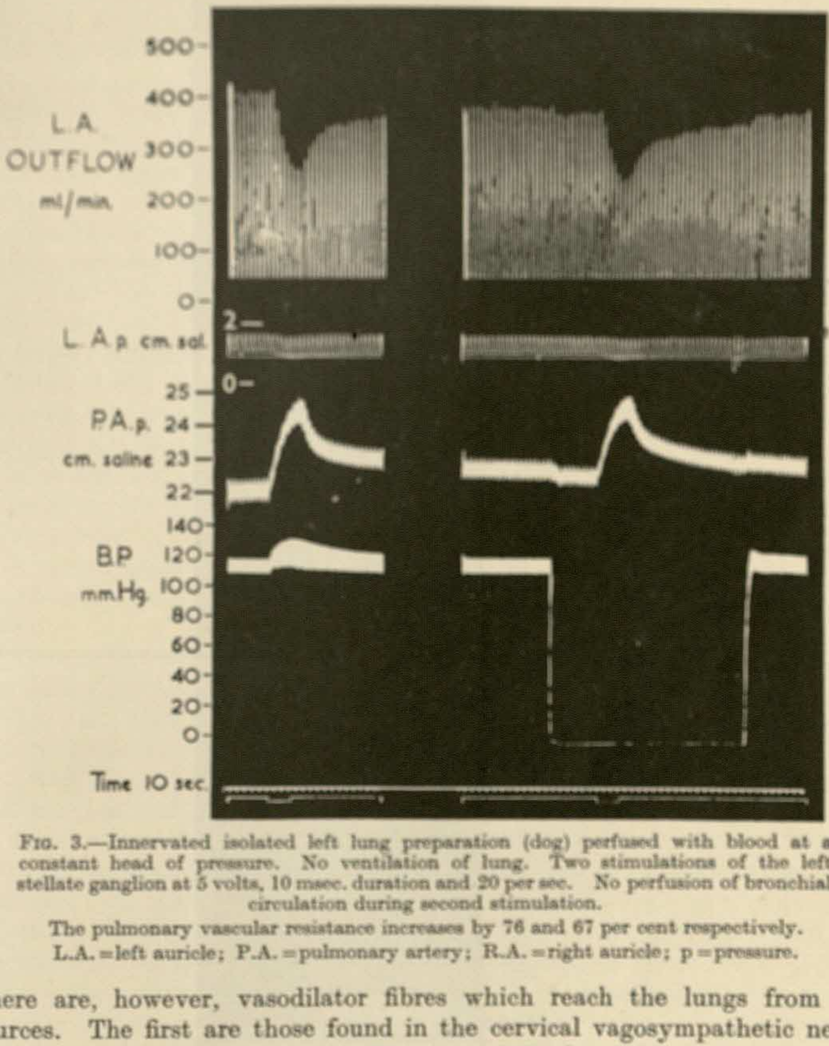


FIG. 3.—Innervated isolated left lung preparation (dog) perfused with blood at a constant head of pressure. No ventilation of lung. Two stimulations of the left stellate ganglion at 5 volts, 10 msec. duration and 20 per sec. No perfusion of bronchial circulation during second stimulation.

The pulmonary vascular resistance increases by 76 and 67 per cent respectively.

L.A. = left auricle; P.A. = pulmonary artery; R.A. = right auricle; p = pressure.

There are, however, vasodilator fibres which reach the lungs from two sources. The first are those found in the cervical vagosympathetic nerves and are paralyzed by atropine. They are difficult to demonstrate because usually they cannot be stimulated without also stimulating the bronchoconstrictor fibres, and if atropine has to be given to eliminate passive bronchoconstrictor effects no vasodilatation will be seen. Occasionally, however, bronchoconstrictor responses are absent and therefore it is unnecessary to administer atropine and in these circumstances stimulation of

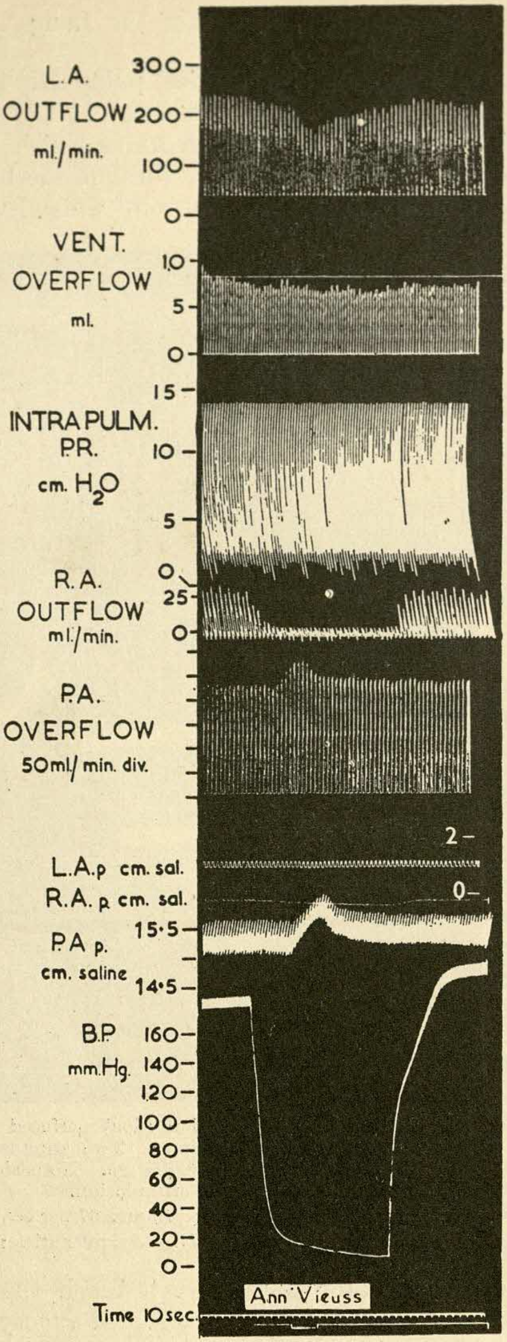


FIG. 4.—Innervated isolated left lung preparation (dog) perfused with blood at a constant head of pressure.

Stimulation of left ventral branch of annulus of Vieussens 5/10/20. No perfusion of bronchial circulation.

Tidal air = 53 c.c. initially; ventilation overflow shows no significant change.

Stimulation increases pulmonary vascular resistance by 35 per cent.

the nerve causes pulmonary vasodilatation (fig. 1B). Attempts to discover whether eserine potentiated this effect failed because nerve stimulations after eserine injections caused bronchoconstriction. Vasodilator fibres of this kind probably have their cells of origin among the vagal nuclei of the medulla; they have not yet been demonstrated under conditions which eliminate passive bronchial vascular system effects and so it cannot be stated with certainty that they activate the pulmonary vascular bed proper. The presence in the cervical vagosympathetic nerve of adrenergic vasoconstrictor fibres from the thoracic sympathetic outflow together with atropine sensitive vasodilator fibres explains why the response to stimulation of this nerve is predominantly vasopressor in atropinized perfused preparations and predominantly vasodepressor in non-atropinized preparations [Daly and Hebb, 1952].

Vasodilator fibres from the second source (the thoracic sympathetic outflow) are not apparently affected by eserine and are atropine-resistant. They follow the same path and have the same cell stations as the sympathetic vasoconstrictor fibres already described. A curious feature is that they are largely confined to the left side of the body [Daly *et al.*, 1949]. In one experiment the interesting observation was made that stimulation of the sympathetic nerves on the left side caused pulmonary vasodilatation and on the right side a pulmonary vasoconstriction (fig. 1C, D). It is possible that these vasodilator fibres may be similar to those responsible for the pulmonary vasodilator response to sympathetic chain stimulation which can be regularly demonstrated in atropinized and ergotoxinized perfusion preparations. Both vasoconstrictor and vasodilator fibres in the cervical and thoracic vagosympathetic nerves cross to the opposite side of the body [Daly and Hebb, 1954].

Thus in the lesser circulation, as in the greater, both vasoconstrictor and vasodilator fibres can be demonstrated experimentally. These results agree in the main with those of Le Blanc and van Wijngaarden [1924], who, in isolated cat lungs, obtained sympathetic vasoconstrictor and vagal vasodilator responses dissociated from bronchomotor effects. It is relevant here to mention that under controlled conditions of lung perfusion the predominant effect of adrenaline and of noradrenaline [Ewing, Atkinson and Seagar, 1955] is vasoconstrictor and in this respect mimics the adrenergic fibre response. On the rare occasions in which adrenaline produces pulmonary vasodilatation it is generally when it has been given in a small dose and administered early on in an experiment, subsequent doses causing vasoconstriction. On the other hand, acetylcholine in physiological doses mimics as one would expect the dilator fibres [Alcock, Berry and Daly, 1935] which on other grounds appear to be cholinergic. The fact that with electrical stimulation of sympathetic nerves vasoconstriction is more readily demonstrated than vasodilatation of the pulmonary circulation is also true of the systemic circulation and is probably not functionally significant in relation to normal conditions. Indeed, it may be that the intrapulmonary vessels which constrict to nerve stimulation have a different site in the vascular bed from those which dilate, and until this point has been cleared up the significance of the two kinds of responses observed cannot be assessed. In this connexion it should be noted that two kinds of vasodilator responses were observed, the one in which the pulmonary arterial pressure fell rapidly and remained down (fig. 1B), the other in which recovery was gradual (fig. 1D). They might possibly denote an action on two different parts of the intrapulmonary blood vessels, alternatively, they might indicate two kinds of vasodilator fibres.

GASEOUS CONTROL OF THE PULMONARY VASCULAR BED

In his Silliman Lectures, Haldane [1922] argued that "It seems probable that by some means at present unknown to us a fair adjustment is maintained normally between air and blood supply. For instance, the muscular walls of bronchioles may be concerned in adjusting the air supply, or the arterioles or capillaries may contract or dilate so as to adjust the blood supply". In the ensuing years some evidence was obtained that both bronchomotor and pulmonary vasomotor activity could take place independently of the central nervous connexions. Curiously enough little notice was taken of the observations of Löhr [1924], Binet and Bargeton [1940] and Binet and Bourlière [1941] that under certain conditions inhaled carbon dioxide increases the pulmonary vascular tonus and it was not until 1946 that interest in the problem was revived by the important discovery of Euler and Liljestrand [1946] [see also Liljestrand, 1948] that hypoxia causes an abrupt increase in pulmonary arterial pressure in chloralosed cats. Inhalation of carbon dioxide mixtures had a similar but smaller effect. Within a few years their results received confirmation from a number of sources. In isolated perfused lung (chiefly cat and dog) experiments, which eliminated the complication of changes in cardiac output, it was shown that a deficiency of oxygen [Logaras, 1947; Nisell, 1948, 1950; Duke, 1951, 1954] or an excess of carbon dioxide [Logaras, 1947; Hebb and Nimmo-Smith, 1948; Duke, 1949, 1956] in the inspired air raises the pulmonary artery pressure. It was further found in dog and rabbit that unilateral breathing of a low oxygen mixture increases the vascular resistance of the hypoxic lung and shunts a greater blood flow through the other lung [Dirken and Heemstra, 1948 *a*, *b*; Attwell, Hickam, Pryor and Page, 1951; Peters and Roos, 1952; Rahn and Bahnson, 1953; Stroud and Rahn, 1953].

There is less agreement as to the effect of hypoxia in man. In the main it would appear that hypoxia increases the pulmonary vascular resistance in normal subjects and also in some patients with respiratory disease [Westcott, Fowler, Scott, Hauenstein and McGuire, 1951; Doyle, Wilson and Warren, 1952] but not in others [Fishman, Himmelstein, Fritts and Cournand, 1955]. This aspect of the problem has been critically reviewed by Lilienthal and Riley [1954] who discuss the complicating factors which may mark any direct effect of hypoxia on the lesser circulation.

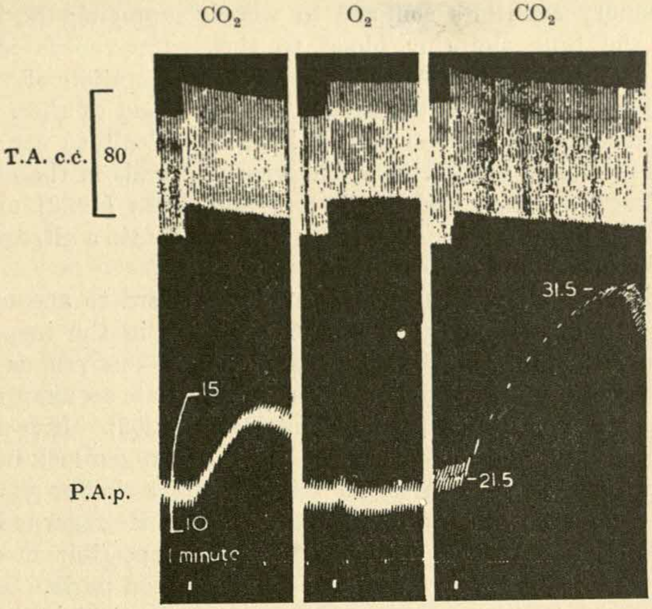
Euler and Liljestrand [1946], also Liljestrand [1948] put forward the attractive hypothesis that if the blood flow becomes inadequate in relation to ventilation in some parts of the lung, the corresponding alveolar air will become richer in oxygen and poorer in carbon dioxide than the rest of the lungs, thus leading to a dilatation of the blood vessels of that part of the lungs with a redistribution of blood as a consequence. It was postulated that the hypoxial rise in pulmonary arterial pressure is due to a local constriction of the pulmonary arterioles confined to vessels supplying the ill-ventilated portions of the lung shunting blood to the better-ventilated parts, the constriction being mediated by the low oxygen saturation of venous blood in the arterioles. But if all the alveoli receive blood of the same gaseous composition a local mechanism of this kind is difficult to conceive, unless one assumes increased sensitivity of the vascular walls in the areas of local hypoxia. This was suggested by Peters and Roos [1952] as a possible mechanism for the reduction in blood flow which occurs in a nitrogen breathing lung while the other lung is receiving oxygen.

Several other explanations have been put forward to account for these gas effects. An oxygen deficiency or CO_2 excess in the inspired air, by producing corresponding changes in the blood on the venous side of the capillaries, may stimulate venous receptors and initiate an axon reflex giving rise to arteriolar constriction [Stroud and Rahn, 1953]. Investigations by Nisell on the cat [1951] suggest that the effect of oxygen lack or CO_2 excess in the inspired air is to constrict the veins, whereas similar gas changes in the pulmonary arterial blood dilate the arterioles. He regarded pulmonary venule constriction in underventilated parts as a possible mechanism for shunting blood by collateral channels through aerated parts. Duke [1954], however, has been unable to confirm the pulmonary arterial dilator effect of oxygen desaturated blood. The problem is further complicated by the findings of Aviado, Cerletti, Alanis, Bulle and Schmidt [1952] on the innervated perfused lung of the dog in which a local dilatation was observed when perfused with blood of low oxygen saturation or when ventilated with a low oxygen gas mixture. The dilatation was associated with anoxia rather than carbon dioxide excess.

It would appear that the variable reports of the effect of oxygen depletion or carbon dioxide excess either in the blood or in inhaled gases can only be explained in part by the response variations in the different animal species. The passive effect on the pulmonary blood vessels of accompanying bronchomotor changes has not been sufficiently controlled in all experiments although it has been shown this plays no part in some experiments (fig. 5). Further information on the effect of oxygen and carbon dioxide on the lung blood volume is required and would be valuable in locating the site of the responsive vessels.

Clinical Investigations

The criteria for the separation of active from passive effects on the pulmonary vascular bed in investigations on man should be no less stringent than those already described for experiments on laboratory animals. The clinical investigator, however, is faced with a more difficult problem because he has not the opportunity for imposing all the required controlled conditions, and the evidence for an active control of the lung blood vessels in man is therefore based on less secure grounds. It has been derived chiefly from patients with cardio-pulmonary disease. A reversible increase in pulmonary arterial pressure without significant changes in cardiac output occurs in response to exercise in cases of severe pulmonary hypertension [Hickam and Cargill, 1948; Bayliss, Ethridge and Hyman, 1950; and others]. There is also a striking rise in pulmonary arteriolar resistance when the "capillary" pressure approximates to the osmotic pressure of the plasma in patients with mitral stenosis and left ventricular failure [Dexter, Dow, Haynes, Whittenberger, Ferris, Goodale and Hellems, 1950]. They suggested that a compensatory arteriolar constriction protects the pulmonary capillaries from a high hydrostatic pressure and pulmonary œdema. A fall in pulmonary arteriolar pressure has been reported following the administration of ganglionic blocking agents in pulmonary hypertensive cases [Fowler, Westcott, Hauenstein, Scott and McGuire, 1950; see also Greene an dBunnel, 1950; Halmágyi,



(1) (2) (3)

FIG. 5.—Macacus rhesus, 4·1 kg., ♀. Isolated right lung perfused at constant volume inflow with heparinized blood of same animal. Observations (1) and (3): at 1 hr. 11 min. and 3 hr. 19 min. after commencing perfusion, 20 c.c. CO_2 added to closed ventilating system; observation (2): at 1 hr. 27 min., 20 c.c. O_2 added to system. Each addition of gas indicated by upward displacement of spirometer (top tracing), as well as by signal [Hebb and Nimmo-Smith, 1948].

Felkai, Iványi, Zsótér, Ténÿi and Szücs, 1953]. The last-named group of workers speculated that receptor bodies in the pulmonary vessels are sensitive to an elevation of pulmonary venous pressure which causes a reflex vasoconstriction of the pulmonary arterioles. Werkö, Frisk, Wade and Eliasch [1951], however, considered that the hæmodynamic changes caused by ganglionic blocking agents were due to pooling of blood in the systemic circulation with a resultant diminution in venous return. Of more than passing interest is that sleep reduces the pulmonary arterial pressure in patients with pulmonary hypertension arising from mitral stenosis or left heart failure [Halmágyi *et al.*, 1953].

Other observations on man have been interpreted as indicating that nervous or humoral mechanisms for pulmonary vascular control exist but there is no general acceptance of this view among clinicians, although Cournand, hitherto the chief protagonist of the passive rôle of the pulmonary vascular bed, stated in 1950 that observations obtained on normal man in his laboratory "have provided new ammunition to those who hold that vasomotor control plays an active rôle in the regulation of pulmonary blood pressures and blood flow".

None of the observations on human subjects so far reported fulfils all the criteria which are essential for the demonstration of an active control. A change in pressure drop across the pulmonary vascular bed even when unaccompanied by alterations in cardiac output is not certain evidence of an active change in pulmonary vascular resistance unless passive effects on that resistance due to alterations in left atrial pressure can be excluded. It is therefore important that information from measurements of left atrial pressure should always be obtained, or some means employed for keeping this pressure constant during observations. The measurement of left atrial pressures by the insertion of a needle directly into the left atrium [Allison and Linden, 1953] is preferable to the wedged catheter method. It may be remarked too that wedged catheter and left atrial pressures may not bear the same relation to one another in normal as compared with pathological lungs in which there is a greater development of pre-capillary and capillary communications between the bronchial and pulmonary vascular systems.

The complexity of active and passive control of the lung blood vessels may be gauged from the diagram in fig. 6 which only illustrates the main mechanisms, but any one of these when operative may lead to a chain reflex activating one or more of the remainder. As an example, the release of a hormone into the blood stream apart from actively and passively affecting the pulmonary blood vessels as shown in the diagram, may, owing to a concomitant change in blood pressure, cause a reflex bronchomotor response [M. de B. Daly and Schweitzer, 1951] leading to further passive effects on the vascular bed. It is no wonder, therefore, that the physiologist in an endeavour to clarify the nature of pulmonary vascular control mechanisms turned from experiments on the whole animal in the laboratory to lung perfusion preparations under controlled ventilation. There seems little doubt that a somewhat similar line of approach will have to be evolved for obtaining precise information on the vascular control in human lungs. Thus in human surgery circumstances may so combine on rare occasions that it is desirable to utilize controlled perfusion and ventilation of a lung lobe as an oxygenator in an assisted circulation system required for intracardiac operations, and also to section the pulmonary nerve supply, either the sympathetic or vagosympathetic, to the perfused lobe. In such circumstances perfusion pressure measurements during the application of a stimulus to the peripheral end of the sectioned nerve in the atropinized subject to prevent unwanted bronchomotor effects would detect whether an active nervous control of the lung vessels was present. A functional test of this kind could reveal whether the intrapulmonary blood vessels are under nervous control, but if positive would not decide to what extent the bronchial vascular and pulmonary vascular systems participate.

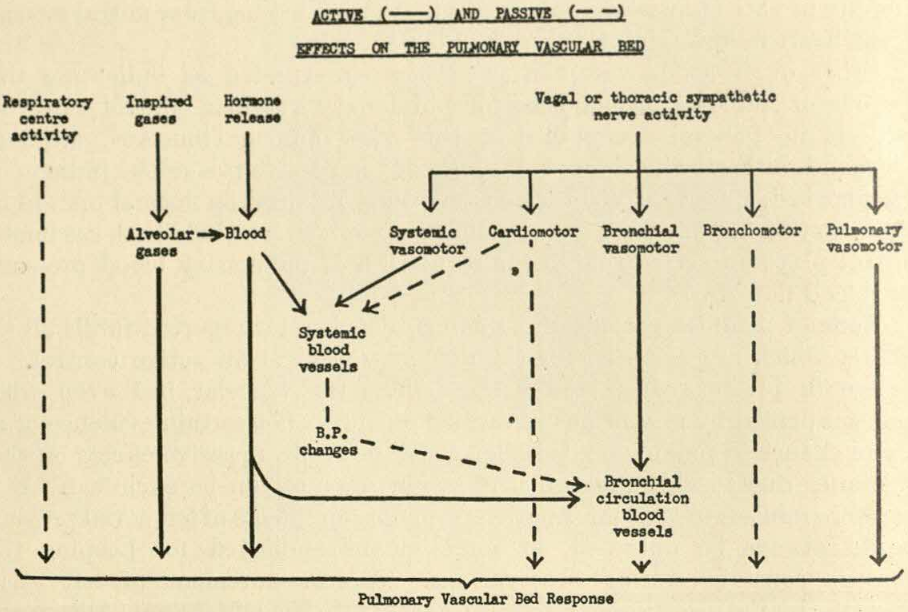

ACTIVE (——) AND PASSIVE (– –)
EFFECTS ON THE PULMONARY VASCULAR BED
Respiratory centre activity
Inspired gases
Hormone release
Vagal or thoracic sympathetic nerve activity
Alveolar gases
Blood
Systemic vasomotor
Cardiomotor
Bronchial vasomotor
Bronchomotor
Pulmonary vasomotor
Systemic blood vessels
B.P. changes
Bronchial circulation blood vessels
Pulmonary Vascular Bed Response

FIG. 6.—Chains of biological events leading to active and passive effects on the pulmonary vascular bed.

There is little or no smooth muscle in the pulmonary arterioles in the lungs of man [Benninghoff, Hartmann and Hellman-Lund, 1930; Benninghoff, 1935; Brenner, 1935] and their activation by nerve stimuli is therefore doubtful yet it should not be forgotten that the muscular arteries of 100–1000 μ in diameter and smaller veins which have a thicker wall than the larger veins [Schmidt, 1953] are potential vascular territories for nervous control. Whether these vessels are supplied with efferent nerve fibres has yet to be established firmly. Larsell and Dow [1933] found fibres ending in relation to smooth muscle cells of the tunica media of the pulmonary arteries which they cautiously stated "probably have their origin from cells in the upper thoracic ganglia of the sympathetic chain . . .".

Of more than passing interest to our problem is the medial hypertrophy of the muscular pulmonary arteries and the development of a media in the arterioles which appear to be manifestations of pulmonary hypertension [Heath and Whitaker, 1955 *a*, *b*; 1956]. That the more suggestive clinical evidence for an active control of the lung vessels has been derived from observations on patients with pulmonary hypertension may be fortuitous, but, on the other hand, it may be related to the increased amount of smooth muscle in the arteries and arterioles which, if associated with a comparable increase in its nerve supply, would realize the potential conditions for a greater active vasomotor nerve control. This speculation has the advantage that the probability of its truth might be tested in two ways. The first by a histological comparison of the nerve supply to normal and pathological blood vessels and the second by comparing the pulmonary vascular resistance responses to pulmonary nerve stimulation in normal and in pulmonary hypertensive subjects under the "ideal" surgical conditions already defined.

The evidence so far presented indicates that investigations have not been carried out under sufficiently controlled conditions to enable any opinion to be expressed as to the nervous control either of the bronchial or pulmonary vascular systems in normal human subjects. Nevertheless, certain observations on pulmonary hypertensive patients suggest that the blood vessels governing the inflow of blood to the pulmonary alveolar capillaries may be controlled by central or intrapulmonary nervous reflexes. Any conclusions from the clinical results presently available must, however, be nebulous in contrast to the greater certainty of deductions which can be and have been drawn from the experimental investigations on the dog.

COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE PULMONARY VASCULAR BED

The thickness and arrangement of muscle in the pulmonary arterial and venous vessels of the lung show marked variations in different animal species [Benninghoff, 1935; Guieysse-Pelissier, 1937; Castigli, 1954] and it would be expected that the more abundant the mural musculature the greater the potentiality for an active vasomotor control. For these reasons the observations reported here on the pulmonary vascular control in the dog cannot be transferred to other animal species. Nevertheless, the clinical problems which have been under discussion might receive clarification from the fields of comparative physiology and pathology. Thus studies on pulmonary hæmodynamics in patients with pathological medial hypertrophy and in laboratory animals which normally possess a thickened media in corresponding parts of the pulmonary vascular bed might reveal common functional characteristics of the control and of the locality in the vascular bed of activated vessels. A fruitful approach also would be the correlation of pulmonary vasomotor nerve responses with histological changes in the pulmonary vascular bed in laboratory animals in which surgical procedures have been undertaken for the simulation of human cardio-pulmonary diseases.

The experimental criteria for the demonstration of active vasomotor responses —to nervous, humoral or gaseous stimuli—should be similar to those already laid down in our discussion on pulmonary vasomotor nerve control. The histological studies would reveal the extent not only of the smooth muscle development in particular vascular sites, but also of the collateral circulation development, of which we already have considerable knowledge from the outstanding investigations of Liebow and his school [1956]. Such comparative studies present a formidable task and demand the co-operation of those interested in different disciplines; they should, however, give an insight into the modifications of pulmonary vascular control by pathological processes.

Pre-capillary anastomoses between the bronchial and pulmonary circulations have been described in healthy lungs of a number of animal species (Table II). Whether they occur in normal human lungs has been debated for many years. The problem requires reassessment in the light of the careful histological studies on normal lungs of man by Hayek [1940, 1942] and Verloop [1948] who found anastomoses in the region of the small bronchi and in certain parts of the visceral pleura. According to Verloop the arrangement of muscle fibres in their walls is extremely complex. These findings are important in that formerly anastomoses were supposed to be solely the result of chronic pulmonary disease.

TABLE II.—BRONCHIAL ARTERY—PULMONARY ARTERY PRE-CAPILLARY ANASTOMOSES IN NORMAL LUNGS

Animal species	Site	
Man	Bronchi and parts of visceral pleura	Hayek, 1940, 1953 Verloop, 1948
,,	Cartilaginous bronchi	Marchand, Gilroy and Wilson, 1950
,,	Not found	Liebow, Hales, Harrison, Bloomer and Lindskog, 1950
,,	,, ,,	Cudkowicz and Armstrong, 1951
Dog	,, ,,	Miller, 1906; Berry *et al.*, 1931
Rabbit	,, ,,	Verloop, 1949
Guinea-pig	Large and small bronchi	,, ,,
,, ,,	Lung hilus	Rakshit, 1949
Rat	,, ,,	,, ,,
,,	Not found	Verloop, 1949
Mouse	,, ,,	,, ,,

The bronchial arteries have both sympathetic and parasympathetic innervation which may extend to the pre-capillary anastomoses and therefore it is possible that the transfer of blood from the bronchial to the pulmonary circulation is under nervous control and these communications may then act as sluices between the two systems. Regarded teleologically, such a controlled communication could operate as a compensatory mechanism in adjusting transient inequalities in output of the two sides of the heart. Hayek [1951] suggested that, since the bronchi and neighbouring alveoli are supplied with branches originating from the anastomoses (fig. 7), the selective closure of one or the other end of the anastomoses may determine whether bronchial or pulmonary arterial blood is supplied to the bronchi. The suggestion has also been made that the high pressure bronchial arterial system may shunt deoxygenated pulmonary artery blood away from poorly ventilated areas under normal [Shedd, Alley and Lindskog, 1951] or pathological conditions [Liebow *et al.*, 1950].

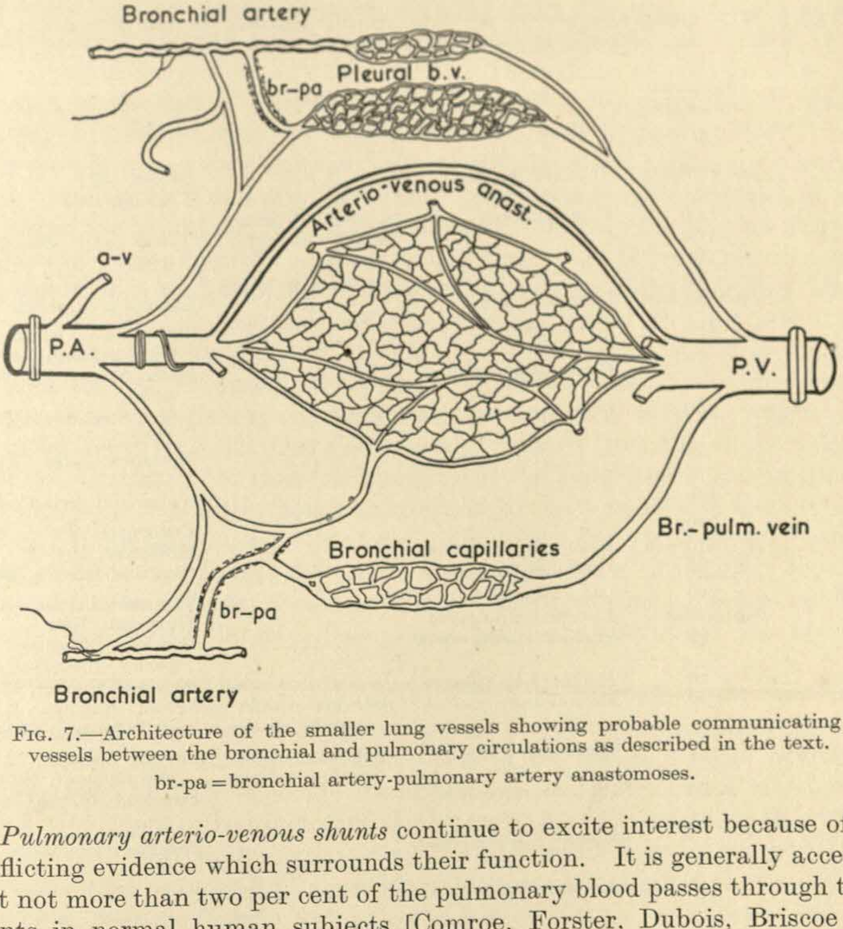


FIG. 7.—Architecture of the smaller lung vessels showing probable communicating vessels between the bronchial and pulmonary circulations as described in the text. br-pa = bronchial artery-pulmonary artery anastomoses.

Pulmonary arterio-venous shunts continue to excite interest because of the conflicting evidence which surrounds their function. It is generally accepted that not more than two per cent of the pulmonary blood passes through these shunts in normal human subjects [Comroe, Forster, Dubois, Briscoe and Carlsen, 1955]. Tobin and Zaraquiey [1950], however, found that glass spheres 500 μ in diameter, which greatly exceed the diameter of the capillaries, pass through the pulmonary vascular bed in normal human lungs examined post-mortem [Table III]. The possibility that the spheres reached the left atrium from the pulmonary artery via some part of the bronchial-pulmonary circulation anastomoses cannot be definitely excluded but if admitted it would still demand the existence of communications of 500 μ diameter between the bronchial arterial and pulmonary venous systems. In the dog a dramatic demonstration of pulmonary a–v shunts has been furnished by Rahn, Stroud and Tobin [1951]. Thoratrast injected through a lateral opening of a catheter wedged into a pulmonary arterial branch was observed by cine-radiography to short-circuit the capillaries and pass rapidly into the left atrium. Normally the thoratrast injected through an unwedged catheter was seen to pass relatively slowly through the pulmonary vascular bed into the left atrium. These investigators were kind enough to run through the

Table III.—Observations on Arterio-venous Shunts in the Pulmonary Circulation of Normal Lungs

Animal species	Maximum diameter	Method	Site	
	μ			
Man	No evidence	Injected lungs	..	Miller, 1937
,,	,,	Histological	..	Verloop, 1948
,,	500	Glass spheres	Lobular divisions	Tobin and Zaraquiey, 1950
,,	100	,, ,,	Smaller bronchi	
,,	200	,, ,,	Pleura	
,,	25	,, ,,	Alveolar sacs	
,,	No evidence	Vinylite casts	..	Liebow *et al.*, 1950
Dog	180	Glass spheres	..	Prinzmetal, Ornite, Simkin and Bergman, 1948
,,	..	Cine-radiography	..	Rahn, Stroud and Tobin, 1950
,,	200	Glass spheres	..	Rahn, Stroud and Tobin, 1950
,,	28*	,, ,,	..	Bostroem and Piiper, 1955
,,	420	,, ,,	..	Niden and Aviado, 1956
Cat	390	,, ,,	..	Prinzmetal *et al.*, 1948
,,	No evidence	Lycopodium spores	..	Hurliman, 1949
,,	> 25	Radioactive spheres	..	Sirsi and Bucher, 1953
Rabbit	290	Glass spheres	..	Prinzmetal *et al.*, 1948
,,	No evidence	Lucopodium spores	..	Hurliman, 1949
,,	19*	Surface tension effect	..	Gordon, Flasher and Drury, 1953
,,	> 25	Radioactive spheres	..	Sirsi and Bucher, 1953
,,	20	Microscopic obs. *in vivo*	Arteriole-venule communications	Irwin, Burrage, Aimar and Chesnut, 1954
Guinea-pig	12	Microscopic obs. *in vivo*	Arteriole-venule communications	Irwin, Burrage, Aimar and Chesnut, 1954
,, ,,	> 25	Radioactive spheres	..	Sirsi and Bucher, 1953
Rat	> 25	,, ,,	..	,, ,, ,, ,,
,,	17*	Surface tension effect	..	Gordon *et al.*, 1953

* Regarded as being probably distended capillaries.

film for me several times and it was indeed a striking demonstration. They also found that glass spheres of 200 μ diameter could pass through the pulmonary vessels. The observations of Irwin, Burrage, Aimar and Chesnut [1954] are of particular interest because they were able to detect blood flowing through pulmonary arteriole-venule shunts in transilluminated lungs of the rabbit and guinea-pig.

It is difficult to reconcile the variable results obtained from the physiological, histological and glass sphere experiments unless it is conceded that they are determined by the physiological conditions of the experiment. The discrepancies might be explained on the grounds that the a-v shunts are closed under normal conditions or even by anxiety evoked by experimental procedures and thus there is little oxygen desaturation of arterial blood. In this connexion Hickam, Cargill and Golden [1948] have drawn attention to the effects of emotional stimuli on the cardio-vascular system and the a-v oxygen difference. The problem is of further interest in relation to pulmonary hæmodynamic changes resulting from the injection of multiple minute emboli into the pulmonary artery because Niden and Aviado [1956] have obtained suggestive evidence that the immediate anoxæmia caused by embolism opens the a-v shunts. Whitteridge [1950] put forward the interesting hypothesis that the impaction of emboli in pre-capillary vessels down stream from nerve endings—stimulation of which evokes an upstream reflex vasoconstriction—may facilitate a continuous vasoconstriction, thus protecting the capillaries from a high pressure. It may also be that the high pressure in the pulmonary arterial vessels central to the reflexly constricted area would open up a-v shunts and serve the same purpose [see Rahn, Stroud and Tobin, 1952]. Alternatively, the stimulation of the afferent nerve endings postulated by Whitteridge may cause a reflex dilatation of a-v shunts. To what extent the pulmonary vasoconstriction evoked by minute emboli is dependent upon intrapulmonary reflexes or central nervous reflexes has yet to be discovered but there is reason to believe from the work of Price, Hata and Smith [1955] that the integrity of the sympathetic innervation of the lungs may be a major factor in addition to the operation of local reflexes [Niden and Aviado, 1956]. Price *et al.* found that provided the sympathetic innervation to the lungs was intact barium sulphate introduced into the pulmonary artery of a Starling heart-lung-head preparation caused pulmonary hypertension which largely disappeared after elimination of the cephalic circulation.

As Lilienthal and Riley [1954] state, "there is small doubt that large potential shunts of several types exist". The requirement now is more knowledge of the conditions under which these shunts become operative. Our survey may fitly conclude by mention of the more recent writings of Hayek [1951] and of Hochrein and Schleicher [1953] who consider that blood passing from the pulmonary artery to the pulmonary veins has three alternative pathways, a high vascular resistance path through the capillaries, a smaller resistance path through the larger pleural capillaries and the smallest resistance through the arterio-venous shunts (fig. 7).

In retrospect, the clinical approach to the problem of pulmonary vasomotor control cannot at the present time cover all the experimental controls necessary for its solution. What is required is a combined operation of clinicians, histologists, pathologists, physiologists and surgeons. There is especially a need for a greater endeavour to perfect methods of staining the nerve fibrils to the lung vessels, a problem which may turn out to be largely one for the histochemist. It is essential that we should know on which parts of the complex system of lung vessels each of the three kinds of stimuli we have considered operate, and also how far the physiological background on which they are superimposed determines the kind of responses they evoke.

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AN INVESTIGATION OF THE PERISTALTIC REFLEX IN RELATION TO ANATOMICAL OBSERVATIONS. By EDITH BÜLBRING, R. C. Y. LIN and G. SCHOFIELD. From the Departments of Pharmacology and Human Anatomy, University of Oxford.

(*Received for publication 31st May* 1957)

Isolated segments of small intestine from guinea-pigs, rabbits and cats were used to investigate the peristaltic reflex. The reflex was unimpaired by extrinsic denervation of the small intestine, but was abolished by each of the following procedures:

(*a*) by asphyxiation of the mucous membrane,

(*b*) by local anæsthesia of the mucous membrane after administration of cocaine into the lumen, and

(*c*) by removal of the mucous membrane.

Combined anatomical and physiological observations are consistent with the view that the intrinsic reflex arc mediating the peristaltic reflex consists of at least two sets of enteric neurons:

(*a*) sensory neurons, the processes of which are distributed to the mucous membrane on the one hand, and to motor neurons on the other, and

(*b*) motor neurons which innervate the muscle coats.

ONE of the most conspicuous features of the anatomy of the innervation of the alimentary tract is the presence of nerve fibres which are distributed to the mucous membrane. Two distinct groups of mucosal nerve fibres can be distinguished in all parts of the gut which contain a ganglionated submucous plexus; one is extrinsic in origin and the other, consisting of the mucosal processes of enteric neurons, is intrinsic in origin [Schofield, 1957 *a*].

Some of the extrinsic mucosal nerve fibres are peripheral processes of cells situated in the cerebro-spinal ganglia; a greater proportion, however, appear to be postganglionic fibres of sympathetic origin which terminate on mucosal blood vessels. The extrinsic afferent fibres in the mucous membrane undergo degenerative changes when the mesenteric nerves supplying a segment of the gut are cut, when the dorsal root ganglia are excised from the level of T6 to L4, and when the vagus nerves are divided. The vagal afferent fibres appear to be restricted mainly to the stomach and adjacent parts of the alimentary tract. Although none of the afferent nerve fibres can be traced to a termination in the muscle coats, many give rise to fine intraganglionic branches as they traverse both the myenteric and submucous plexuses to terminate in the mucous membrane.

In normal animals, it is possible to distinguish mucosal nerve fibres with a smaller diameter than that of the extrinsic fibres, and these arise from neurons situated in the submucous plexus. The intrinsic fibres can be identified more certainly, however, when all extrinsic fibres disappear after dividing the mesenteric nerves supplying a segment of small intestine.

It is generally assumed that extrinsic nerves are not primarily involved in the peristaltic reflex, and that the reflex arc is entirely intrinsic. Nevertheless, stretch receptors or tension receptors, the discharge of which has been recorded from the vagus nerve [Paintal, 1954 *a*, *b*; Iggo, 1955, 1957], may still be involved, as well as endings of sensory fibres running in the sympathetic trunks. As the ganglion cells from which these fibres arise are situated outside the intestinal wall, extrinsic denervation causing nerve degeneration would thus abolish the peristaltic reflex. Experiments of this kind have been performed by various workers [for references see Babkin, 1950; Alvarez, 1950; also Gregory, 1946, 1947] and have led to the conclusion that the sensory neurons are probably situated in the intestinal wall itself. However, not all workers who have investigated motility have strictly distinguished between peristalsis and intestinal contractions. Furthermore, neither the site at which the reflex is initiated, nor the exact neural pathways by which it is mediated, have been established with certainty.

The present investigation was therefore undertaken to answer three main questions:

(*a*) Is the peristaltic reflex initiated by means of sensory nerve endings situated in the mucous membrane?

(*b*) Do the extrinsic nerve fibres, which terminate in the mucous membrane, mediate the peristaltic reflex by means of intraganglionic collateral branches, even when severed from their parent neurons?

(c) Is the peristaltic reflex dependent entirely upon intrinsic neural pathways?

METHODS

Anatomical.—Neural tissue in specimens of intestine was demonstrated with the use of a silver impregnation method evolved by Schofield [1957 *b*]. This technique outlines both normal and degenerating nerve fibres consistently and selectively. Normal specimens of gut in each animal species used for the purposes of this investigation were stained and used as controls. Specimens were also taken from segments of gut in animals subjected to various denervation experiments and the presence of nerve degeneration in them was confirmed.

Physiological.—Isolated loops of small intestine of guinea-pigs, rabbits and cats were used. The peristaltic reflex was tested by the presence or absence of the response to an increase in intraluminal pressure. The response consisted of co-ordinated contractions of both the longitudinal and circular muscle layers, propelling the contents in a cephalo-caudal direction.

Two recording methods were used: (1) that described by Trendelenburg [1917] modified by Feldberg and Lin [1949] and (2) a modification in which not only the contractions of the intestinal muscle but also the volume of the propelled contents could be recorded, using apparatus similar to that described by Gregory [1950] and by Streeten and Vaughan Williams [1951].

For guinea-pig's ileum Tyrode solution was used containing 8·0 g. NaCl, 0·2 g. KCl, 0·2 g. $CaCl_2$, 0·01 g. $MgCl_2$, 0·05 g. NaH_2PO_4, 1·0 g. $NaHCO_3$, 1·0 g. glucose per litre. For both rabbit's and cat's small intestine a modified solution [Bülbring, 1953] was found more suitable; it contained 7·8 g. NaCl, 0·35 g. KCl, 0·28 g. $CaCl_2$, 0·01 g. $MgCl_2$, 0·165 g. NaH_2PO_4, 1·37 g. $NaHCO_3$, 1·4 g. glucose per litre. Both solutions were equilibrated with 95 per cent O_2 and 5 per cent CO_2 at 37° C.

In order to determine the effect of degeneration of extrinsic nerves on the peristaltic reflex, animals were operated under ether anæsthesia up to ten days before the experiment was carried out. Guinea-pigs were used for the removal of dorsal root ganglia (T4 to L5), for sectioning the splanchnic nerves and for dividing the mesentery attached to segments of small intestine 10–20 cm. in length. Cats were used for removal of the cœliac ganglia and for sectioning the vagi.

Both normal and operated guinea-pigs, rabbits and cats were used to obtain isolated preparations in which the mucosa had been removed. For this purpose a loop of intestine was turned inside out by slipping it over either a glass rod or rubber tubing while it was immersed in Tyrode solution at 37° C. The mucosa was gently scraped off, using the back of a scalpel or filter-paper or a brush, and then the loop of intestine was returned to its normal position.

RESULTS

1. *Normal Animals*

Anatomical Observations.—Enteric neurons which give rise to well-defined processes may be classified [following Dogiel, 1896] according to the length of their processes as Type I, Type II or Type III cells. However, they may also be classified into two groups: either multipolar cells with long and short processes (fig. 1) or unipolar and bipolar cells (fig. 2); and this may prove to have a functional, as well as a morphological, significance. Although there is as yet no evidence that only unipolar and bipolar cells are concerned in carrying afferent impulses to multipolar cells, it is certain that many of their processes are distributed to the mucous membrane (fig. 3) and others terminate in myenteric ganglia. On the other hand, the processes of some of the multipolar cells have been traced into the muscle coats (fig. 4) and they presumably carry motor impulses to them.

Physiological Observations.—The guinea-pig's ileum showed little pendular activity: but co-ordinated waves of peristalsis, *i.e.* contractions moving from the oral to the caudal end of the loop, were easily elicited by raising the inside pressure to about 3 cm. water. The response was usually clear-cut and reproducible over many hours. Contractions in response to the addition of acetylcholine and small doses of nicotine to the bath were recorded as controls in most experiments to test the viability of the muscular coats and the enteric ganglia. It was found that the peristaltic reflex as well as the response to nicotine was unimpaired after a loop of guinea-pig intestine had been left for 24 hr. in an isolated organ bath and adequate oxygenation had been ensured by a slow flow of Tyrode solution through the bath, part of which flowed through the lumen of the intestine. If, however, the lumen of the

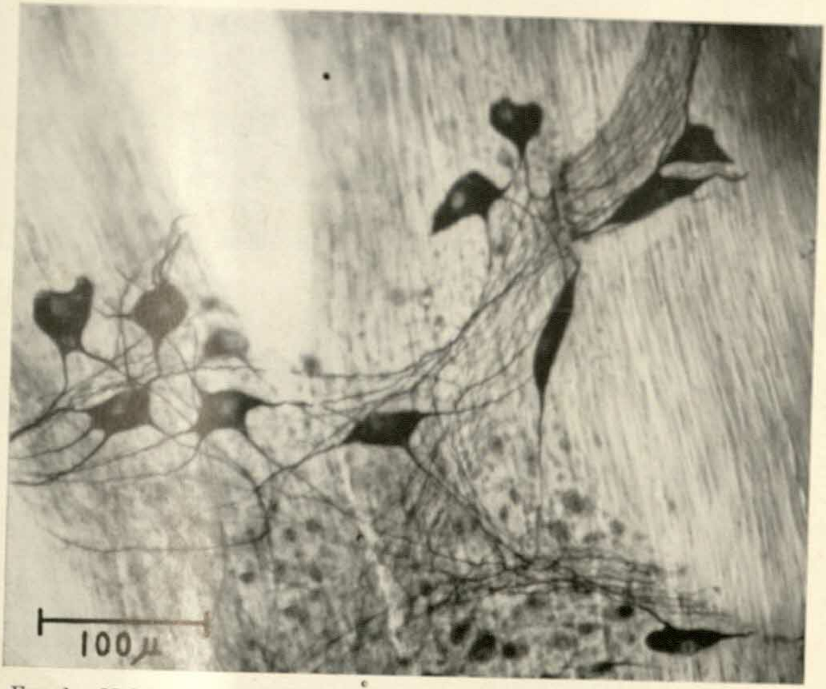


FIG. 1.—Multipolar neurons from the myenteric plexus of the jejunum of a cat.

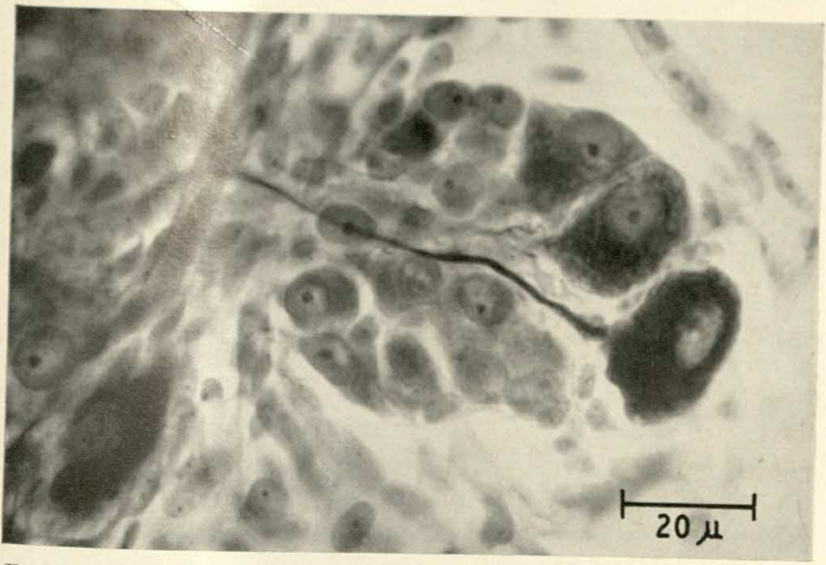


FIG. 2.—A submucous ganglion from the jejunum of a cat. The process of the darkly stained unipolar cell was found to bifurcate a short distance from the ganglion; one of the branches could be followed towards the mucous membrane and the other towards the myenteric plexus.

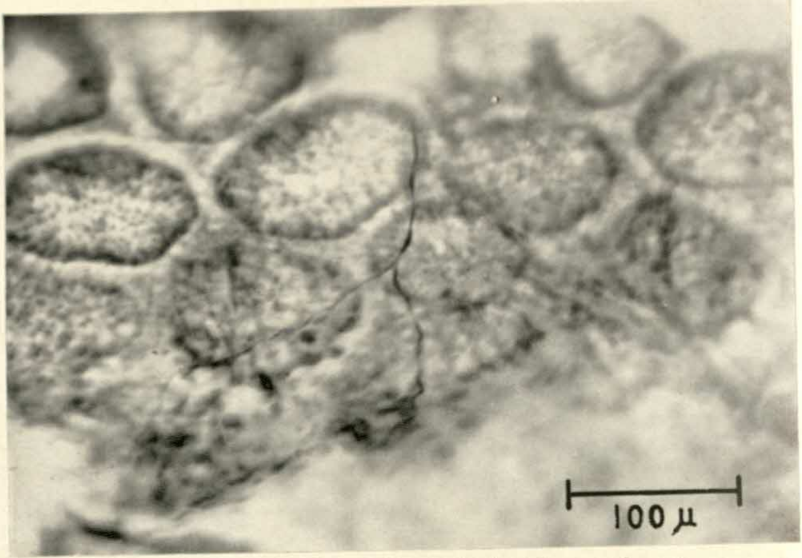


FIG 3.—A mucosal nerve fibre from the jejunum of a cat.

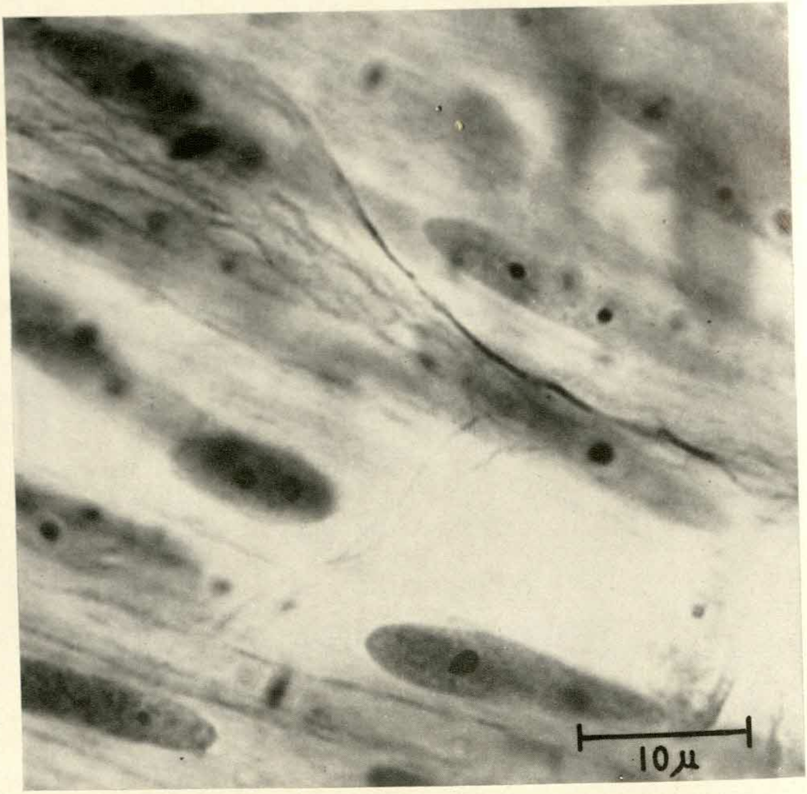


FIG. 4.—Bundles of nerve filaments in the longitudinal muscle coat of the jejunum of a rat. Although the filaments do not anastomose, they simulate the appearance of a reticulum when aggregated in bundles and examined in one focal plane.

intestine was not constantly bathed in this manner the peristaltic reflex was absent after 24 hr. It appeared that asphyxiation of the mucous membrane abolished the reflex, or possibly autolysis or accumulation of toxic material.

Loops of rabbits' intestine showed very strong pendular activity. As soon as the intraluminal pressure was raised to about 4 cm. H_2O peristaltic contractions were superimposed. In the kymograph record this was shown by the contractions becoming bigger in amplitude and less frequent, and the peristaltic waves could be clearly seen by observing the loop itself.

In loops of small intestine taken from the cat a higher inside pressure of 8–10 cm. H_2O was found to be necessary to elicit the peristaltic response. An increase in the KCl content in the solution up to twice the normal amount was found to facilitate the peristaltic reflex so that it could be recorded more readily.

2. *The Effect of Extrinsic Nerve Degeneration*

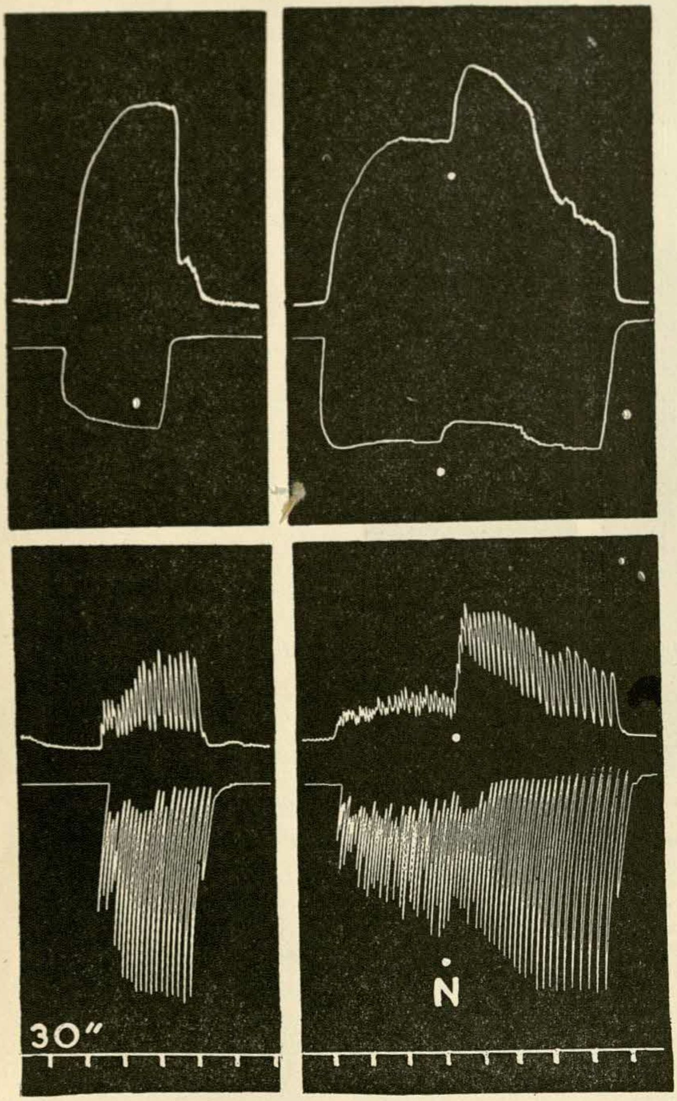
Anatomical Observations.—Degenerative changes were observed in the enteric plexuses of all animals subjected to extrinsic denervation of the gut. The remarkable rapidity with which these changes developed confirmed previous observations [Schofield, 1957 *c*]. The first change was axonal swelling which began within 6 hr. after nerve section and was characterized by increased argyrophilia. The axon next became varicose, then fragmented so that after 12 hr. the degenerating fibres consisted of argyrophilic particles arranged in series. The nerve fibre remnants persisted in this form for 24 hr., after which they progressively disappeared. However, some large argyrophilic fragments persisted for at least 4 days after operation.

Physiological Observations. (i) *Effect of Removing Dorsal Root Ganglia.*—In 2 guinea-pigs the dorsal root ganglia were excised on both sides from T4 to L5. The peristaltic reflex in an isolated loop of ileum was normal 24 and 48 hr. after the operation.

(ii) *Effect of Sectioning the Splanchnic Nerves.*—In 2 guinea-pigs the splanchnic nerves on both sides were exposed through dorsal incisions close to the last rib and sectioned. One was killed 72 hr. and the other 144 hr. later, and the peristaltic reflex of a segment of ileum was found to be as strong as a normal one.

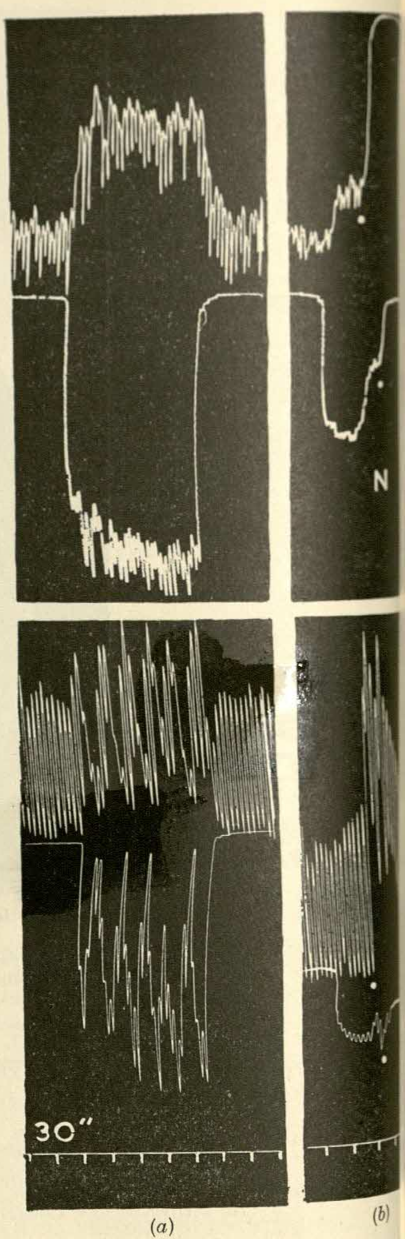
(iii) *Effect of Removing the Cœliac Ganglia.*—Two cats were operated under ether anæsthesia by a midline abdominal incision. The cœliac ganglia on both sides were exposed and removed. Both cats recovered from the operation in good condition. The peristaltic reflex of an isolated segment of the jejunum of both cats was tested 8 days after the operation and it was as strong as a normal one.

(iv) *Effect of Dividing the Vagus Nerves.*—In 2 cats both vagi were cut below the diaphragm. Both recovered in good condition. The peristaltic reflex was tested in a piece of duodenum and in a piece of jejunum 7 and 10 days respectively after the operation. Both segments from both animals exhibited a powerful peristaltic reflex.



(*a*) (*b*)

FIG. 6.—Guinea-pig ileum. Records as in fig. 5. Upper tracing taken from a segment after the removal of the mucous membrane. Lower tracing from adjacent normal segment. (*a*) Response to raising intraluminal pressure to 3 cm. H_2O; (*b*) response to nicotine (N), 0·2 mg. in 150 ml. bath, at a subthreshold intraluminal pressure of 2 cm. H_2O.



(*a*) (*b*)

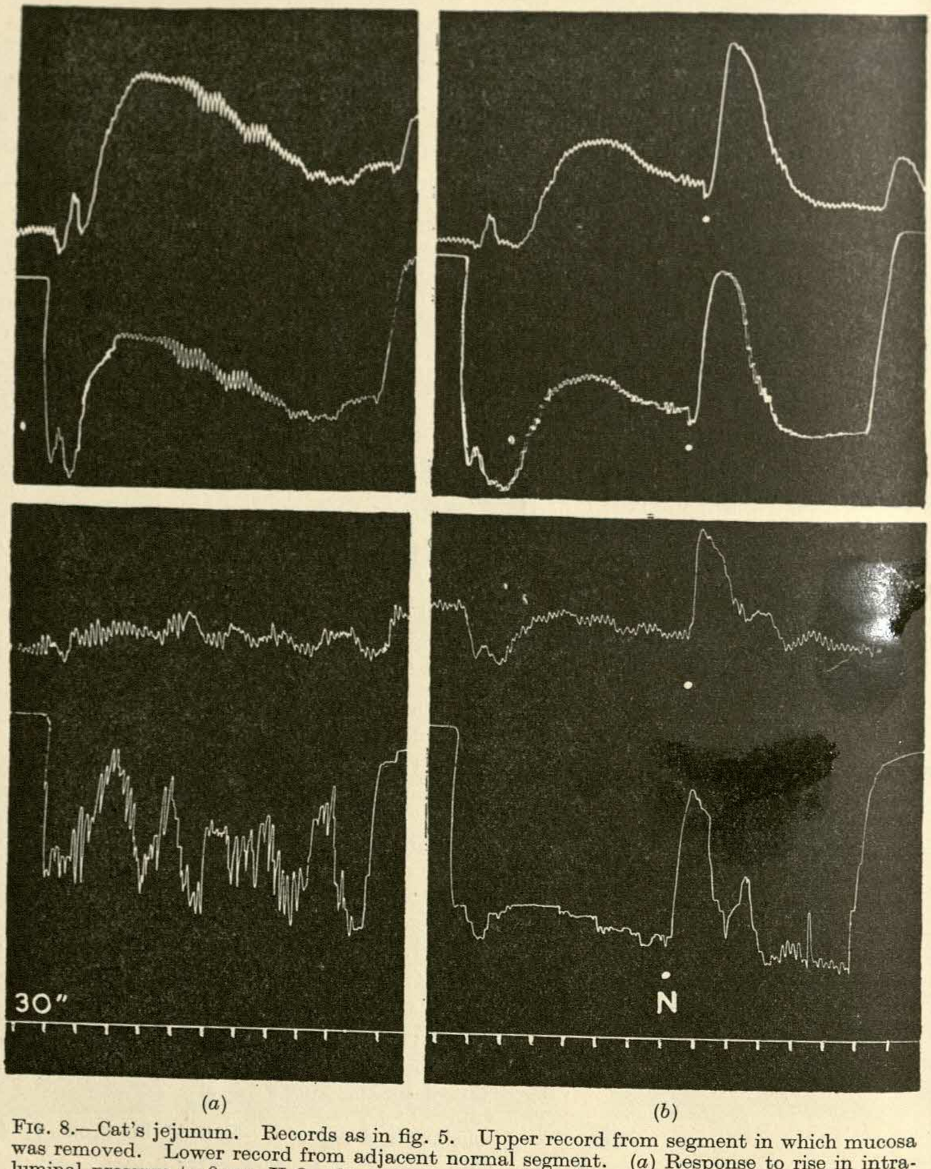
FIG. 7.—Rabbit's jejunum. Records
fig. 5. Top tracing from a segment
removal of mucosa, bottom tracing fr
adjacent normal segment. (*a*) Respons
rise in intraluminal pressure to 3 cm.
(*b*) response to nicotine (N), 0·2 m
150 ml. bath, at a subthreshold intralu
pressure of 1 cm. H_2O.

compared with that of an adjacent normal piece (lower tracing). The peristaltic reflex was entirely absent while the response to nicotine was as powerful as that of the normal part. Similar experiments were carried out on segments of the small intestine from rabbits and cats. Fig. 7 shows clearly the difference between the response of a segment from rabbit's intestine deprived of its mucosa (upper tracing), and that of the normal segment (lower tracing). While pendular activity continued in the upper record, it gave way to slow peristaltic waves in the lower record. Nevertheless, the response to nicotine was equally vigorous in both preparations. Fig. 8 is taken from an experiment on the cat's jejunum. In the loop in which the mucous membrane had been removed (upper tracing) both muscle coats responded to the raised pressure with sustained contractions and, superimposed, an increased pendular activity. This response was entirely different from the sequence of slow peristaltic waves occurring in the normal loop. In order to record the response to nicotine the intralumen pressure was only raised to a level which was subthreshold for eliciting peristalsis; both segments responded with similar contractions. It should be mentioned that in this cat the cœliac ganglia had been removed 8 days previously without, however, abolishing normal peristalsis.

Table II.—Effect of removing the Mucous Membrane on the Peristaltic Reflex

Experimental procedure	Animal species	Peristaltic reflex	Response to nicotine
Segment turned inside out	Guinea-pigs	Reduced in 4 out of 5 exp. Normal in 1 out of 5 exp.	..
Segment turned inside out, and mucosa removed	Guinea-pigs	Abolished in 4 out of 4 exp.	..
Segment turned inside out, then returned to normal position	Guinea-pigs	Normal in 4 out of 5 exp. Reduced in 1 out of 5 exp.	Normal in 5 out of 5 exp.
Segment turned inside out, mucosa removed, and then returned to normal position	Guinea-pigs	Abolished in 6 out of 8 exp. Reduced in 2 out of 8 exp.	Normal in 8 out of 8 exp.
	Rabbits	Abolished in 7 out of 8 exp. Reduced in 1 out of 8 exp.	Normal in 8 out of 8 exp.
	Cats	Abolished in 4 out of 4 exp.	Normal in 4 out of 4 exp.

All the results obtained after removal of the mucous membrane are summarized in Table II together with the control experiments described above. Out of a total number of 24 experiments removal of the mucosa abolished the peristaltic reflex in 21, leaving the response to nicotine intact. In 3 experiments the peristaltic reflex was not entirely abolished though much reduced. It is evident from the records, that in some preparations in which the mucosa was removed, or cocaine was applied, muscular contractions occurred (figs. 7, 8 and 9) in response to raising the intraluminal pressure. Not only the records but also the direct observation of the preparation showed that these contractions were different from peristalsis, but this could only be proved by measuring the volume of fluid transported. Though the



(*a*) (*b*)

FIG. 8.—Cat's jejunum. Records as in fig. 5. Upper record from segment in which mucosa was removed. Lower record from adjacent normal segment. (*a*) Response to rise in intraluminal pressure to 8 cm. H_2O; (*b*) response to nicotine (N), 0·2 mg. in 150 ml. bath, at a subthreshold intraluminal pressure of 3 cm. H_2O.

majority of the experiments were carried out using the method devised by Trendelenburg [1917], a few further experiments were therefore done with a modified method in which the propulsion of the intestinal contents could be measured. It was found that loops of intestine without mucosa were entirely unable to transport fluid when the intraluminal pressure was raised.

Anatomical Observations.—Histological observations carried out on specimens which were used for the physiological experiments and deprived of all but the deepest layers of their mucous membrane showed that, in some, this procedure had led to extravasations of blood in the region occupied by neurons of the submucous plexus. In other specimens the cell bodies of the neurons were apparently unaffected by the removal of the mucous membrane. It may be concluded that in some preparations the whole of the afferent side of the neuronal arc may have been injured while in others only the sensory endings were removed.

4. *The Effect of Cocaine on the Peristaltic Reflex*

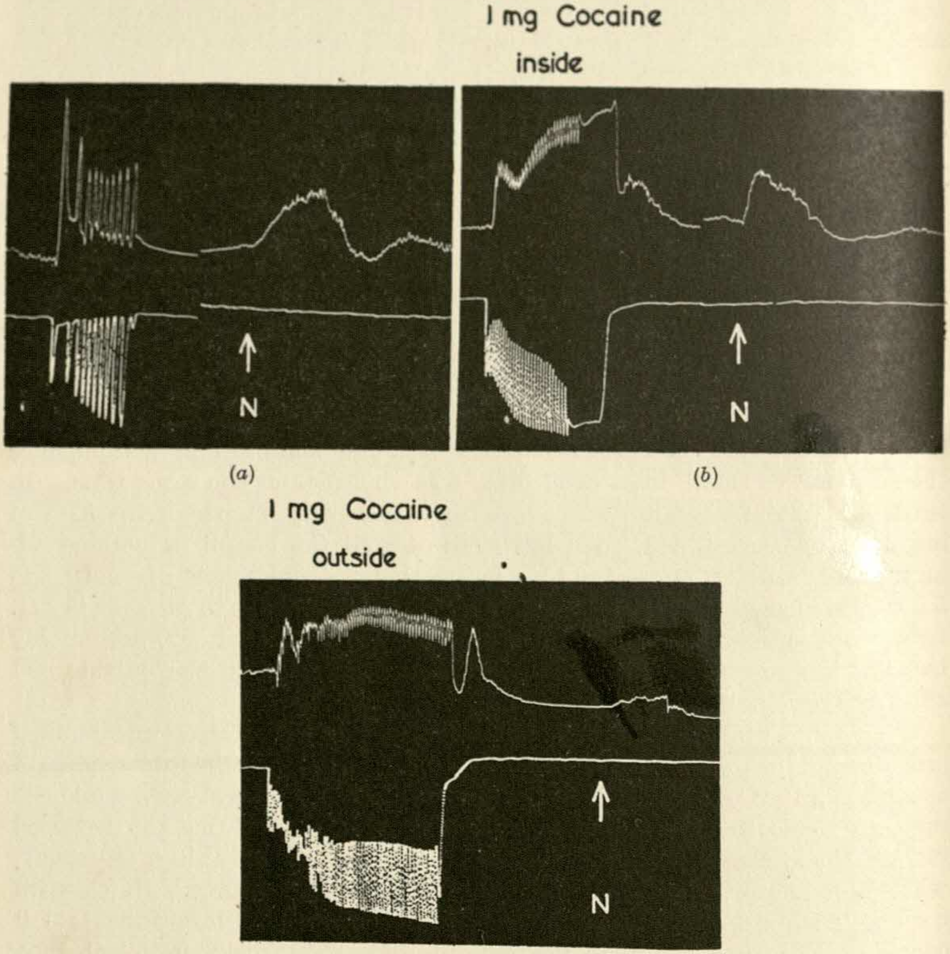
Feldberg and Lin [1949] showed that cocaine in low concentrations (4×10^{-6}) abolished the peristaltic reflex by a ganglion-blocking action, since the response to small doses of nicotine was abolished at the same time. In their experiments cocaine was added to the solution in which the loop of intestine was suspended. We have now tested the action of cocaine administered into the lumen, and we found that concentrations of 1×10^{-4} to 5×10^{-4} abolished the peristaltic reflex leaving the response to nicotine intact. In most experiments this differential action was only transitory, presumably because the cocaine gradually diffused from the inside to the outside and affected the ganglia.

Fig. 9 shows in (*a*) the normal peristaltic response to increased intraluminal pressure and the response to nicotine $7{\cdot}5 \times 10^{-5}$. In (*b*), after the introduction of cocaine 5×10^{-4} into the lumen, the peristaltic reflex was absent, while the response to nicotine was still present. It should be noticed that the increased intraluminal pressure produced a burst of small contractions at high frequency, which were, however, distinctly different from peristaltic waves. In (*c*), when cocaine in a lower concentration was added to the outside solution these small contractions were even more pronounced, but peristalsis and the nicotine contraction was abolished.

DISCUSSION

One of the findings in a recent investigation of the anatomy of the innervation of the gut under normal and experimental conditions was that intrinsic neural pathways exist by means of which nerve impulses originating in the mucous membrane could reach the muscle coats. Morphological investigations of these pathways suggest that they involve at least one synaptic interruption, and this view is supported by the well-known fact that the peristaltic reflex is abolished by large doses of nicotine, which block ganglionic transmission.

It has always been assumed that the reflex arc was entirely intrinsic and our experiments have confirmed what was first shown by Langley and Magnus [1905] that degenerative section of the mesenteric nerves neither impairs nor abolishes the peristaltic response. It has also been known that a rise of intraluminal pressure above a certain threshold was the stimulus eliciting the response [Trendelenburg, 1917], but no information has been available as to the site of the pressure receptors. In the present investigation an attempt was made to determine the extent to which sensory impulses originating in the mucous membrane played a part. The response to tactile stimuli of the mucosa has been studied by Raiford and Mulinos [1934] who came to the conclusion that nervous connexions existed between the mucosa and the muscle coats, and that the contractions were due to a true local reflex which



(a) (b)

(c)

Fig. 9.—Guinea-pig's ileum. Records as in fig. 5. Responses to raising the intraluminal pressure and to nicotine (N) 0·2 mg. in 150 ml. (*a*) Normal; (*b*) after introducing 1 mg. cocaine into the lumen; (*c*) after adding 1 mg. cocaine to the fluid in the bath.

could be abolished by the application of cocaine to the mucosal surface. Gregory [1950] observed that the transport of fluid through extrinsically denervated loops of intestine was increased if the fluid was acid or hypertonic; and he found that this local response was abolished by applying 5 per cent procaine to the mucous membrane. In the present investigation we studied strictly the peristaltic response to increased intraluminal pressure. We found that while the peristaltic reflex was unaffected by degenerative changes in the extrinsic nerve fibres, the reflex could be abolished by procedures which affected the integrity of mucosal nerve fibres. It was possible to eliminate the sensory impulses originating in the mucous membrane by asphyxiating the mucous membrane, by applying a local anæsthetic and by removing almost the entire thickness of the mucous membrane.

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AFFERENT SYSTEMS CONVERGING UPON CEREBELLAR PURKINJE CELLS IN THE FROG.

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The activity of single cells in the cerebellum, presumed to be Purkinje cells, have been recorded extracellularly with micropipettes in anæsthetized frogs. The cells were spontaneously active, firing either single impulses or short high-frequency bursts of impulses. The single impulses started with a rapid prepotential followed by a diphasic spike of up to 35 mV peak to peak. The bursts started with a slower prepotential and consisted of a repetitive discharge, lasting up to 50 msec., of spikes of diminished amplitude and of isolated prepotentials, occasionally accompanied by a slight persistent negativity. Inactivation responses of the type described by Granit and Phillips [1956] in the cat have been seen in one frog only.

Stimulation within the lateral part of the cerebellum with single cathodal shocks from a second microelectrode could excite either of these two responses alone, in the same form as they occurred spontaneously. The burst had a relatively constant latency of about 3 msec., which was unaltered by previous firing of the cell. The single spike was excited either with a short and relatively constant latency (antidromic or monosynaptic activation) or with a long and variable latency (polysynaptic activation). These three different responses could be selectively excited by altering the strength of stimulation or by adjusting the position of the stimulating microcathode, and it is suggested that they are produced by the activity of separate pathways which converge on the Purkinje cell.

In the cerebellar cortex of decerebrate cats, Granit and Phillips [1956, 1957] recorded, with extracellular micropipettes, single diphasic action potentials whose amplitude sometimes reached 50 mV peak to peak. These "giant spikes" were shown to be due to single Purkinje cells, for their response to stimulation of the fastigial nucleus was sometimes obtained with so short a latency that it could be explained only by antidromic excitation of the Purkinje axons. The form of the giant spike was always the same, whether arising naturally or in response to shocks to the fastigial nucleus or to the cerebellar cortex. It began with a prepotential wave in which the microelectrode became positive with respect to the reference electrode. This wave steepened, at an obvious point of inflection, into the positive-going phase of the diphasic spike. These potential changes were considered to be due to the flow of membrane current at the region of contact with the cell exterior. Their magnitude implied a large recording resistance. In extracellular recordings from cerebellar and other neurones of the frog, Müller and Larremendi [Müller, personal communication] have shown that the growth of the giant spike is actually associated with an increase in the recording resistance.

A characteristic reaction of cat Purkinje cells was a wave of depolarization, lasting 15–30 msec., occurring either spontaneously or in response to shocks,

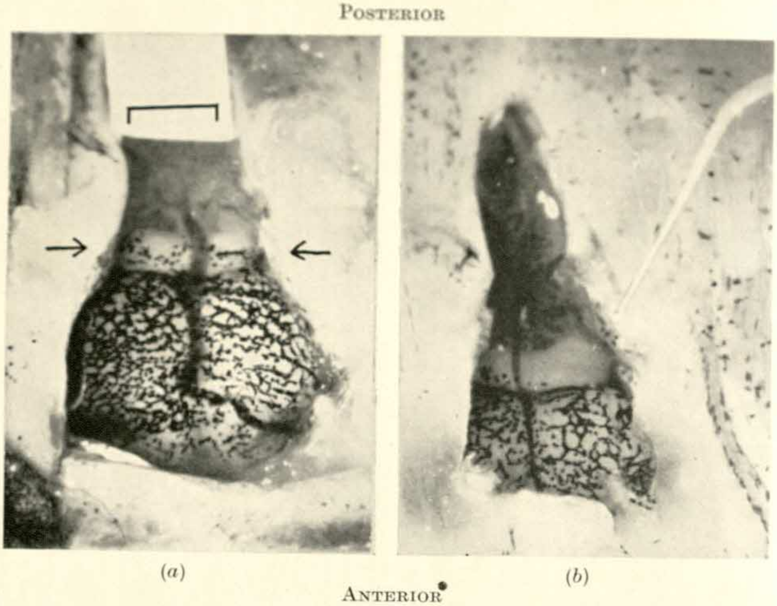

FIG. 1.—(*a*) Optic lobes (flecked with pigment) and cerebellum (marked by arrows) of *Rana temporaria*, viewed from above. Bracket spans 1·0 mm. (*b*) Another preparation, showing silver filled glass capillary introduced into lateral aspect of cerebellum in readiness for stimulation. The recording microcapillary (not shown) explores the cerebellum lying between the stimulating capillary and the midline vein.

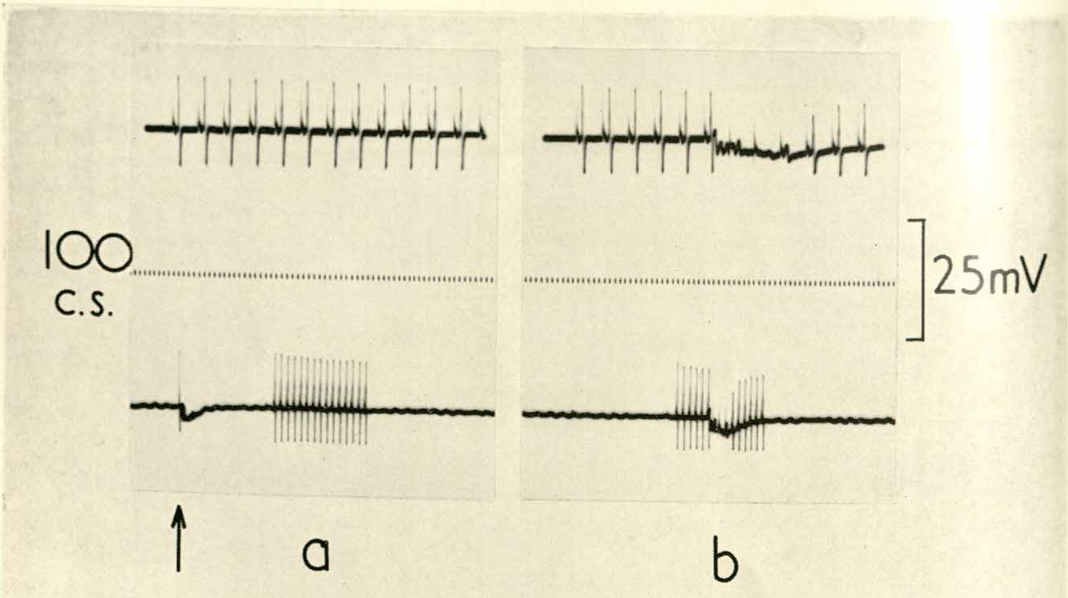

FIG. 5.—(*a*) Spontaneously occurring inactivation response (marked by arrow) and repetitive stimulation of Purkinje cell at 80 c/s. (*b*) Another inactivation response incident during a similar period of repetitive stimulation. Time scale 100 c/s for continuous records. Shock artifacts (80 c/s) indicate sweep velocity.

and followed by a pause in the rhythmic natural discharge of giant spikes. This was called the "inactivation response" [Granit and Phillips, 1956]. It appeared to be a new type of inhibition mediated by excessive depolarization. Because of the unique synaptic relationship between basket cells and Purkinje cells, it was tentatively suggested that this specific reaction was the effect on the Purkinje cell of basket cell activity. Unfortunately, intracellular recording proved difficult in the cat's cerebellum and the giant spike and inactivation responses could not be further investigated.

In the hope of further understanding of these processes, we have investigated the frog's cerebellum by similar methods. Extracellular giant spikes have been readily recorded, but frog Purkinje cells have proved no easier to impale than those in the cat, and no new light has been cast on the nature of the giant spikes. According to Larsell [1923] and Glees, Pearson and Smith [1958] there are no basket-like endings in the frog's cerebellum, and therefore this preparation might have provided a crucial test of the origin of the inactivation response. In fact, only one frog Purkinje cell has shown a few inactivation responses of the type occurring so frequently in every Purkinje cell record from the cat. The other frog Purkinje cells have shown brief high frequency bursts as well as single giant spikes. There is evidence that the giant spike and the high-frequency burst are fired by separate afferent systems converging upon the Purkinje cell.

METHODS

Frogs weighing about 20 g. were anæsthetized by injection into the subcutaneous lymph space of 2·0 mg. of soluble hexobarbitone in 1 per cent solution. It was sometimes necessary to inject a further 0·5 mg. after 30 min., but usually by that time reflexes were absent. The cerebellum was then exposed by opening the dorsal aspect of the foramen magnum with fine scissors, avoiding, under stereomicroscopic vision, the large venous channels to each side of it. The pia was opened by tearing apart with two watchmakers' forceps, without damaging the cerebellar capillaries or the choroid plexus. During the experiment the narcosis would lighten until brisk limb reflexes returned, but still no respiratory movements. Exploration was continued until the appearance of the first spontaneous limb movements, when the frog was killed.

Experiments with decerebrate frogs were abandoned because of movement. Preliminary experiments with decapitated heads mounted in oxygenated Ringer's solution were abandoned because of the small amount of electrical activity in the cerebellum as compared with that in the lightly narcotized preparation.

Control of temperature was not attempted. The frogs were taken from a chamber maintained at 3° C. and the experiments performed in a room at about 20° C. There was an unknown amount of local radiant heating of the cerebellum from the microscope lamp (although a heat absorbing filter was used to reduce this).

Fig. 1 shows the frog's cerebellum forming a narrow bridge spanning the fourth ventricle, and lying immediately behind the much larger optic lobes. In its cellular organization it resembles a single folium of higher forms. The molecular layer lies along its anterior margin, the granular layer along its posterior margin, and the bodies of the Purkinje cells form a single lamina midway between. Their dendrites are orientated in parasagittal planes within the molecular layer, and at right angles to the transversely running axons formed by the bifurcation of the axons sent into the molecular layer by the granule cells. According to Larsell [1932]. "Climbing fibres are present in typical relation to the Purkinje cell dendrites" [but *cf.* Glees, Pearson and Smith, 1958]. The Purkinje axons pass laterally in the granular layer, some to end in the nucleus cerebelli at the lateral extremity of the organ, which is the forerunner of the cerebellar nuclei of higher vertebrates; others pass through the nucleus to unknown destinations in the neuraxis. Many afferent fibres also pass through the same region. It is therefore the locus of choice for both antidromic and orthodromic stimulation.

Stimulation was by single shocks delivered through a silver-in-glass microcathode with a tip diameter of 15 μ (see fig. 1*b*). The silver barely projected from the glass. The anode was the earthed silver plate on which the frog lay. By varying current strength, or by making small adjustments of the independent micromanipulator on which the stimulating microelectrode was mounted, different assortments of outgoing and incoming fibres could be stimulated, and the effects on single Purkinje cell potentials could be recorded.

Potentials were recorded with glass micropipettes filled with 3 M KCl or 4 M NaCl solution, connected through a cathode follower to a D.C. amplifier. The most satisfactory pipettes had a D.C. resistance of about 10 megohms. The potentials were displayed, at the same amplification, on both beams of a double beam oscillograph; one beam was driven by a time base while the other was not. Photography on moving paper thus gave both a continuous record of the natural discharges of the cell and high speed records of its responses to stimulation. Fuller details of the recording technique are given by Phillips [1956] and Granit and Phillips [1956]. The operative field in the frog is too small to admit mechanical devices to restrain pulsation, but this was not unduly troublesome in small frogs.

RESULTS

Nineteen giant spikes were investigated, and fifteen of these were successfully stimulated.

Natural Activity.—Microelectrode probing of the frog's cerebellum yields spikes of varying configurations and low voltages, many of which may be attributed to injury. The posterior border (granular layer) shows most activity. Cells are often penetrated but the initial membrane potential is low and declines rapidly, and this is associated with high-frequency injury discharge. In the cat, slow-positive-going intracellular potential waves with one or more humps [D potentials of Granit and Phillips, 1956] were of common occurrence after penetration of a cell, and were independent of the deteriorating spike potentials. We have only once recorded small potentials of this type in the frog.

In searching for giant spikes it was found best to make a series of punctures separated by about 20 μ and running along parallel anteroposterior lines. These spikes were usually encountered about midway between the anterior and posterior borders of the cerebellum. It was easy to pick up several in every experiment with amplitudes up to 5 mV peak to peak, but the larger examples we wished to record were more difficult to obtain. As in the cat, it seemed that some specially favourable location of the electrode tip was necessary, and in successful cases, progressive increase in spike amplitude occurred spontaneously, without further adjustment of the micromanipulator. Sometimes one could by-pass the cell on the inward journey, but usually any inward movement of the electrode punctured the membrane and rapidly destroyed the cell. The maximum peak-to-peak voltage in our experiments was 35 mV. The D.C. potential was zero or, at most, a few mV negative, indicating an extracellular recording site. Contact with the cell was usually maintained until terminated by a movement of the frog. It was maintained in one experiment for 4 hr., without decline in spike amplitude. This long survival of a giant spike shows that diffusion of KCl from the microelectrode is not a harmful factor. Similar records obtained with microelectrodes filled with 4 M NaCl show that KCl is not a necessary condition for the recording of giant spikes. It will be assumed that these giant spikes, in the frog as in the cat, signal the discharge of impulses by single Purkinje cells.

If anæsthesia is too deep, natural activity is depressed, and giant spikes are seen only in response to shocks. In lighter anæsthesia they occur in trains at low frequencies (3–5 c/s), varied by spells of silence. As the microelectrode approaches a cell after a diphasic spike is encountered, there is apt to be a slight transient increase in frequency each time the microelectrode is moved closer, as if the cell had mechanoreceptor properties [*cf.* Alanis and Matthews, 1952].

Our continuous records of natural Purkinje cell discharges have usually contained a few examples of a brief high-frequency repetitive burst of rather stereotyped form. These bursts are infrequent—sometimes only one to about every hundred giant spikes—and occur unpredictably, so that they can best be visualized on oscilloscope sweeps by using the amplifier output to start the sweep. They are not obtained from deeply narcotized frogs. It is impossible to be sure whether they have any effect on the rhythm of the natural discharge of giant spikes. Often the burst is followed by a pause in the discharge of spikes; but careful study of many records shows plenty of examples of similar pauses without preceding bursts. The natural rhythms are so irregular that nothing can be proved.

Figs. 2, 3 and 4 illustrate the results described so far. In all records, positivity is signalled by upward deflections, and the upper record of each pair is a single oscillographic sweep, the lower being part of a continuous tracing made by the same amplifier output fed onto a stationary spot and recorded on moving paper. Fig. 2*a* shows one giant spike and fig. 2*b* shows two giant spikes and one repetitive burst recorded from a single Purkinje cell. By rare good fortune this repetitive response happened to fall on a sweep. Characteristically, the onset of the giant spike's prepotential is steeper than that of the repetitive response, as shown on sweeps in (*a*) and (*b*) respectively; and the first spike of the repetitive response goes more positive and less negative than the corresponding peaks of the single giant spike, as can readily be seen in the continuous record of fig. 2*b*. The continuous record also shows a small residual negativity outlasting the repetitive activity.

Repetitive responses have been encountered in two main varieties, illustrated by fig. 3 from another cell. The initial positive phases are not seen on the sweeps, having been used to trigger the time base. Following the negative peak of the initial deflection, there is, in both records (fig. 3*a* and 3*b*), a positive-going wave (marked by an arrow) suggestive of an isolated prepotential. This is followed by a series of prepotentials and spikes with variable delays between onset of prepotential and onset of spike. In fig. 3*a* the amplitude of the successive deflections declines, whereas in fig. 3*b* their amplitude tends to recover. The ensuing pauses in natural firing (continuous records) could be matched by many similar pauses without preceding bursts.

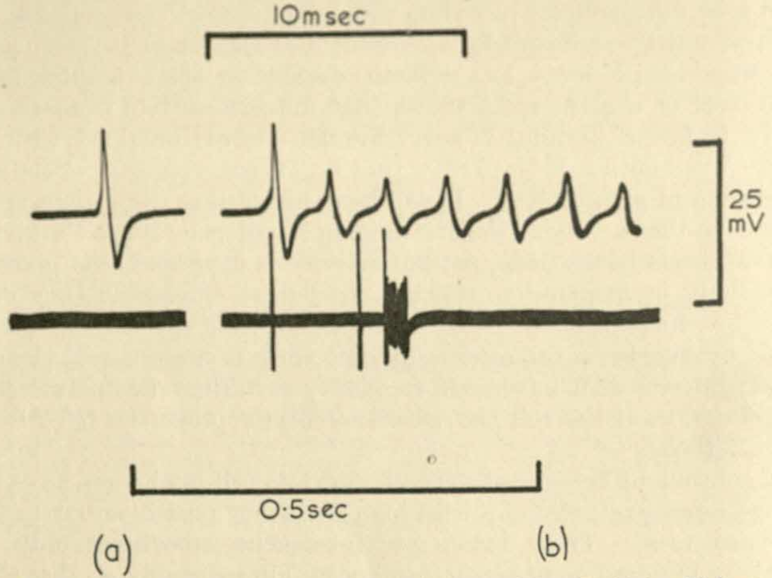

FIG. 2.—Natural discharges of a single unit (presumably a Purkinje cell) from cerebellum of lightly anæsthetized frog. In this and later illustrations, amplifier output is visualized at same amplification on sweeps (upper record of each pair) and continuous record (lower of each pair). Microelectrode positivity gives upward deflection. *Calibrations:* above, 10·0 msec. for sweeps; below, 0·5 sec. for continuous record; right, 25 mV. (*a*) Single giant spike. (*b*) Continuous record shows two giant spikes and one burst. The first part of this burst happened to fall on the sweep. (Original records traced for reproduction as line drawing.)

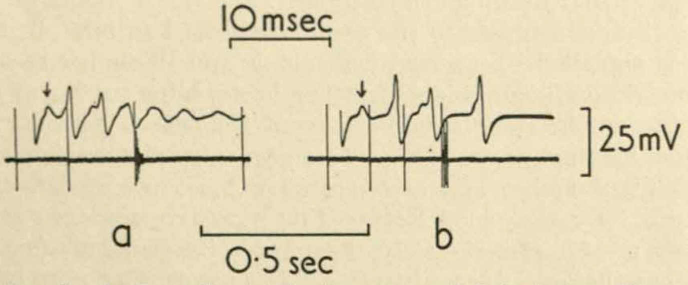

FIG. 3.—Another cell. (*a*) Continuous record (below) shows three giant spikes and one burst. The sweep (above) was triggered by this burst. Sweep begins with first negative-going component of burst, followed by an isolated prepotential (marked by arrow) and a series of waves of declining amplitude. (*b*) A burst of similar onset but recovering amplitude. *Calibrations:* above, 10·0 msec. for sweeps; below, 0·5 sec. for continuous records; 25 mV. (Original records traced for reproduction as line drawing.)

No persisting negativity is seen. Fig. 4, from another cell, shows a repetitive response which closely follows a giant spike. In this example the repetitive activity shows a greater decline in amplitude, and is followed by negativity persisting for about 150 msec.

In one cell only out of nineteen have we seen inactivation responses of the type found by Granit and Phillips [1956] in the cat. This is shown in fig. 5*a* and marked by an arrow. Its initial phase is a positive-negative spike, and this is followed by a negative going wave lasting about 100 msec. The natural firing of this cell was too infrequent to reveal any effect of this wave upon it. But by good fortune one such wave happened to occur during a period of repetitive stimulation at 80 c/s (fig. 5*b*). The cell did not respond to the three shocks incident during the rising phase of the wave. During the falling phase the shocks again became effective and the spikes recovered progressively in amplitude. A control tetanus at the same frequency is shown in fig. 5*a*.

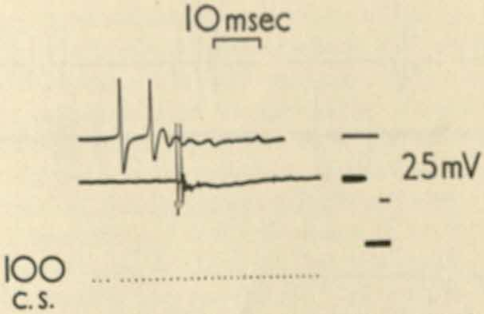

FIG. 4.—Another cell. A giant spike is closely followed by a burst. This burst shows oscillations of diminishing amplitude. Note, in continuous record, persisting negativity lasting over 150 msec. *Calibrations:* above, 10·0 msec. for sweep; below, 10·0 msec. for continuous record; right, 25 mV. (Original records traced for reproduction as line drawing.)

Responses to Micro-Stimulation within the Lateral Region of the Cerebellum.—With suitable placing of the stimulating microcathode, single shocks may elicit, separately or in various combinations: (1) a negative-going wave; (2) a giant spike with a short latency, suggesting antidromic or monosynaptic excitation; (3) alternatively, or in addition to (2), a giant spike with longer and variable latency, suggesting excitation over a polysynaptic pathway; (4) a repetitive burst resembling those occurring naturally.

A sequence of single shocks at 0·6–1·0 sec. intervals also disrupts the prevailing natural impulse rhythm. After stimulation is stopped, there may be silence for 10–20 sec. before natural firing is resumed.

In the experiment illustrated by fig. 6, the stimulating microelectrode remained throughout at the same point in the lateral part of the cerebellum, and the different responses obtained were determined partly by small adjustments of stimulus strength and partly by the natural fluctuation of excitability during the period of observation.

Fig. 6*a* shows the minimum response, a small negative wave, which was recorded alone in 42/278 trials. Such waves were also recorded in other experiments (not illustrated). The amplitude of these waves was not "all-or-nothing", but was graded in relation to stimulus strength. Amplitude was unaffected by an immediately preceding spontaneous giant spike, but the second of a pair of waves elicited by paired shocks suffered reduction at short intervals. This suggests that the negative wave is a massed potential due to the activity of many units, and is not a response of the cell in contact with the recording electrode.

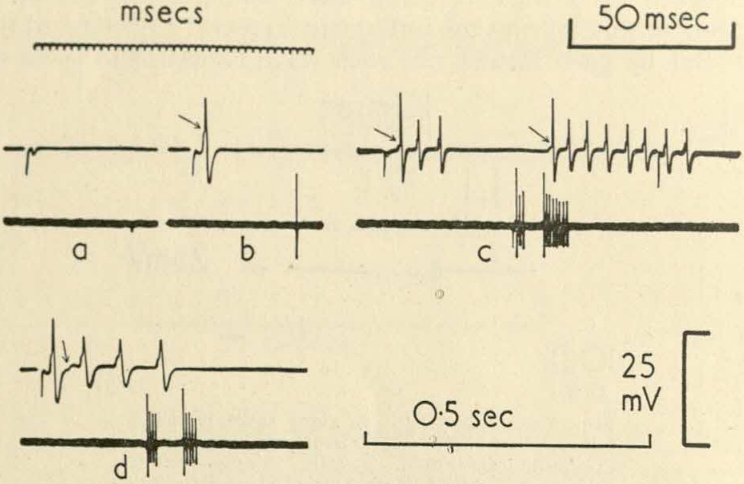

FIG. 6.—Another cell. Responses to microstimulation within the lateral part of the cerebellum. (*a*) Shock, just below threshold for this cell, fires a negative wave due to neighbouring activity. (*b*) Stronger shock fires a single giant spike. Arrow marks point of inflection, at which spike arises from prepotential. (*c*) Shock fires a burst. Note longer latency and different inflection (marked by arrow). A second, longer burst follows the first. (*d*) Shock fires a giant spike followed by a burst. Arrow marks onset of burst's prepotential. *Calibrations:* upper left, 1·0 msec. for sweeps (*a*), (*b*) and (*d*); upper right, 50 msec. for sweep (*c*); bottom right, 0·5 sec. for all continuous records, and 25 mV. (Original records traced for reproduction as line drawing.)

Fig. 6*b* shows the response to slightly stronger stimulation—a giant spike arising just after the crest of the negative wave. In many measured examples, the interval between the shock and the foot of the positive-going prepotential was 1·3–1·8 msec.; the spike arose from the prepotential after a further 0·3–0·4 msec., at a point of inflection which is marked by an arrow.

Fig. 6*c* shows a short repetitive burst excited in the same cell by another shock, followed by a longer burst. The sweep was slower than in (*a*) and (*b*), but the different mode of onset of the response is easily seen. The burst was preceded by a positive prepotential wave which began late—4·5 msec. after the shock. This wave had only about one-third of the amplitude of the prepotential of the ordinary giant spike. It continued for 1·0 msec., then suddenly gave rise to the initial positive-going component of the burst without any further obvious inflection on the upstroke to correspond with that seen at the take-off of the ordinary giant spike. The same characteristic slow prepotential (also marked with an arrow) is to be seen in the first spike of the longer burst occurring later on the same sweep.

In the commonest response to a single shock, the burst was preceded by a typical giant spike (fig. 6*d*). In its mode of onset, this response precisely resembled the mode of onset of the giant spike of fig. 6*b*, and differed in all respects—in latency to foot of prepotential, and in amplitude and duration of prepotential—from the opening phases of the burst response illustrated in fig. 6*c*. Close inspection showed that in all these "spike-and-burst" responses, the delayed, small-amplitude prepotential wave of the uncomplicated burst could be clearly identified about 4·5 msec. after the shock. Its onset at this characteristic latency is marked by an arrow in fig. 6*d*.

This observation suggested that the cell of fig. 6 was being influenced by the shock by volleys converging upon it in separate pathways: one pathway setting up a large amplitude prepotential of brief latency (1·3–1·8 msec.), leading in 0·3–0·4 msec. to the discharge of single impulse; the other pathway giving rise, after a longer latency (4·0–4·5 msec.), to a prepotential of smaller amplitude, leading in 1·0 msec. to the discharge of a burst. The first pathway was excited alone by weaker shocks (fig. 6*b*, 74/278 trials), but the threshold difference was very small, and more often both pathways were thrown into action together (fig. 6*d*, 160/278 trials). Very rarely (2/278 trials), the second pathway was excited without the first (fig. 6*c*).

Separation of these two components by alteration of stimulus strength being difficult, another analytical device was employed. The "single impulse pathway" was made unresponsive by a preceding impulse, and the "burst pathway", which was unaffected by this preceding impulse, then acted in isolation. Naturally occurring giant spikes were used to trigger the sweeps, and also to send in, with variable delay, shocks strong enough to excite a single impulse followed by a burst (fig. 7*a*). Below a critical interval the initial giant spike was blocked and the burst response was thus isolated (fig. 7*b*). Arrows mark the foot of the burst's prepotential wave in both these records. In all the isolated bursts (*e.g.* fig. 7*b*), the initial burst spike arose from the summit of the low prepotential wave, with a doubtful inflection on its upstroke. In the combined spike-and-burst responses (fig. 7*a*), the first spike of the burst was stunted, and arose about a millisecond later, from the declining phase of the prepotential wave. On the upstroke of this spike a decided inflection can be seen.

Experiments on other cells have confirmed that the burst response has its characteristic latency whether or not the shock has also elicited an initial giant spike. Fig. 8*a* shows an early giant spike (latency 0·45 msec.) and fig. 8*b*, with a stronger shock, an early spike followed by a burst (latency 3·1 msec.) whose oscillations first decline in amplitude but then recover. In fig. 8*c* the shock was preceded by a natural impulse which has blocked the early spike. In this case the latency of the burst was 2·8 msec. It shows declining oscillations. The natural burst responses of this same cell (not

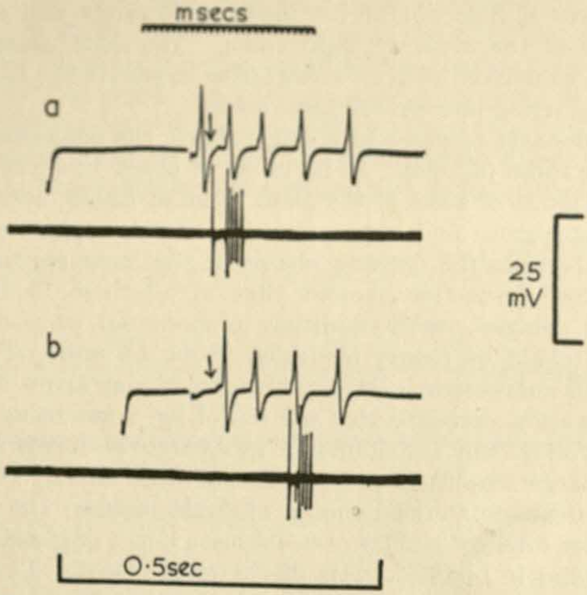

FIG. 7.—Same cell as fig. 5 showing method of separation of spike from burst. Sweeps triggered by natural giant spikes. Shock delivered at different interval from onset of sweep. (*a*) Shock fires giant spike and burst. Burst's prepotential marked by arrow. (*b*) At shorter interval, the initial spike of the response is blocked, and the burst is isolated. Arrow marks its prepotential. *Calibrations:* above, 1·0 msec. for sweeps; below, 0·5 sec. for continuous records; right, 25 mV. (Original records traced for reproduction as line drawing.)

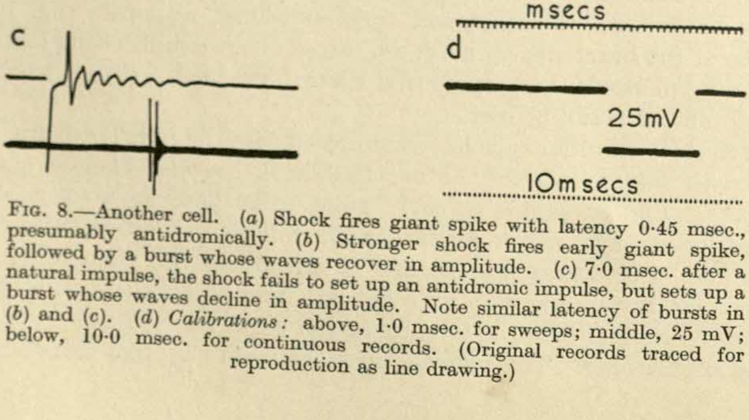

FIG. 8.—Another cell. (*a*) Shock fires giant spike with latency 0·45 msec., presumably antidromically. (*b*) Stronger shock fires early giant spike, followed by a burst whose waves recover in amplitude. (*c*) 7·0 msec. after a natural impulse, the shock fails to set up an antidromic impulse, but sets up a burst whose waves decline in amplitude. Note similar latency of bursts in (*b*) and (*c*). (*d*) *Calibrations:* above, 1·0 msec. for sweeps; middle, 25 mV; below, 10·0 msec. for continuous records. (Original records traced for reproduction as line drawing.)

figured) occurred also in "recovering" and "declining" forms. Because the early spike arises from the shock artefact, its prepotential is obscured, but other records from this cell confirm that its slope was steeper than that of the prepotential leading to the burst.

Figs. 6 and 8 have shown examples of giant spikes fired at short latency by the shock. The shorter latency (0·45 msec.) is suggestive of antidromic excitation of the cell, the longer (1·3–1·8 msec.) might mean antidromic or monosynaptic excitation. The latency in these early responses varies within fairly narrow limits. It cannot be lengthened very much by a preceding impulse. In another cell (fig. 9), the usual latency was 0·8 msec., as seen in the response to the first of each pair of shocks. A second shock 10 msec., after the first (top record) always excited, but with latency lengthened to 1·1 msec. At the critical interval of 7·0 msec., the second shock excited in 4/6 trials with still longer latency (1·4–1·7 msec., middle record), and failed in 2/6 trials (bottom record). At 6·5 msec. (not figured) re-excitation failed in 7/7 trials. Gross delay in take-off of the spike from the prepotential, leading to failure of the spike with isolation of the prepotential, has not been seen.

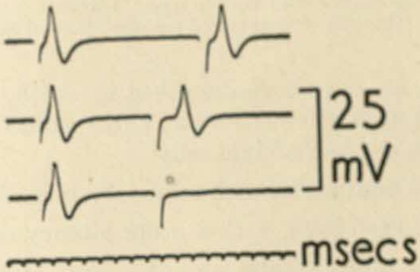

FIG. 9.—Another cell. Effect of paired shocks on short-latency pathway. The first shock of each sweep fires a spike at short latency (0·8 msec.). *Upper sweep:* interval between shocks 10·0 msec. Second shock fires, but with latency (measured to foot of prepotential) lengthened to 1·1 msec. At 7·0 msec. interval, latency of response to second shock was increased to 1·4–1·7 msec. in 4/6 trials (middle sweep), and the second response failed in 2/6 trials (bottom sweep). At 6·5 msec. interval (not shown), response failed in 7/7 trials. *Calibrations:* below, 1·0 msec.; right, 25 mV.

In contrast with the relatively narrow time-limits within which early (antidromic or monosynaptic) excitation has to take effect if it is not to fail, the latency range of delayed (polysynaptic) excitation is more variable. Fig. 10*a* shows an impulse fired at 4·5 msec. latency, 150 msec. after a natural impulse. The shock of fig. 10*b* fell 75 msec. after a natural impulse and the latency of the response was lengthened to 9·4 msec. In the third record (fig. 10*c*) a natural impulse 50 msec. before the shock prevented the polysynaptic response.

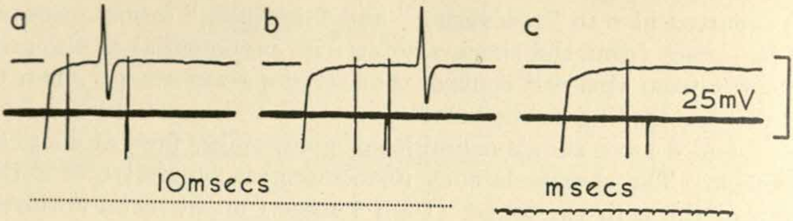

FIG. 10.—Same cell as fig. 7, showing latency variation of polysynaptic pathway. Weaker shocks firing impulses with longer and variable latency. (*a*) Shock incident 150 msec. after a natural impulse: latency of response 4·5 msec. (*b*) Shock 75 msec. after natural impulse: latency 9·4 msec. (*c*) Shock 50 msec. after natural impulse: no response to shock. *Calibrations:* bottom left: 10·0 msec. for continuous records; bottom right: 1·0 msec. for sweeps; extreme right: 25 mV. (Original records traced for reproduction as line drawing.)

Thus far, experiments have been described in which, without altering the position of the stimulating microelectrode, three different excitatory effects have been produced in single Purkinje cells:

(1) Single impulses fired early, with relatively inflexible latency;

(2) single impulses fired later, with a wider latency range; and

(3) bursts with an intermediate latency. Fig. 11 summarizes this experience in the collected responses of a single cell. The ordinate measures the latency of response. Single impulses (open circles) were fired early, with a latency hardly varying around 0·5 msec., or alternatively later, with a latency range of 4–12 msec. These delayed or polysynaptic responses had a lower threshold than the early impulses. The burst response (filled circles) needed stronger shocks, its characteristic latency was around 3 msec., and it was usually but not always preceded by an antidromic impulse which did not affect this latency. The effect of preceding natural impulses on the latency of the three types of response to stimulation is also illustrated. The abscissa of fig. 11 measures the interval between each shock and the impulse preceding it. (Often there was no natural impulse in the 0·6 sec. interval between successive shocks: the circles to the right of the figure refer to this situation.) Clearly the latency of the early impulse is not influenced by its phase relationship to the natural cycles of impulse generation. The same is true of the burst. On the other hand, the polysynaptic pathway is sensitive to the effect of a previous impulse. In it, the latency tends to lengthen as the shock follows the natural impulse more closely, and the circles at the top of the figure show that transmission tends to fail at intervals below 90 msec.

There is further evidence that these alternative modes of activation—early giant spike, delayed giant spike, and repetitive burst response—are due to stimulation of different pathways converging on the cell. If the stimulating microcathode is cautiously moved inwards and outwards along the same track, the recording electrode remaining in contact with the cell, alternative responses can be selectively stimulated. For example, in one experiment, strong stimulation at a depth of 0·465 mm. had no effect on the cell under

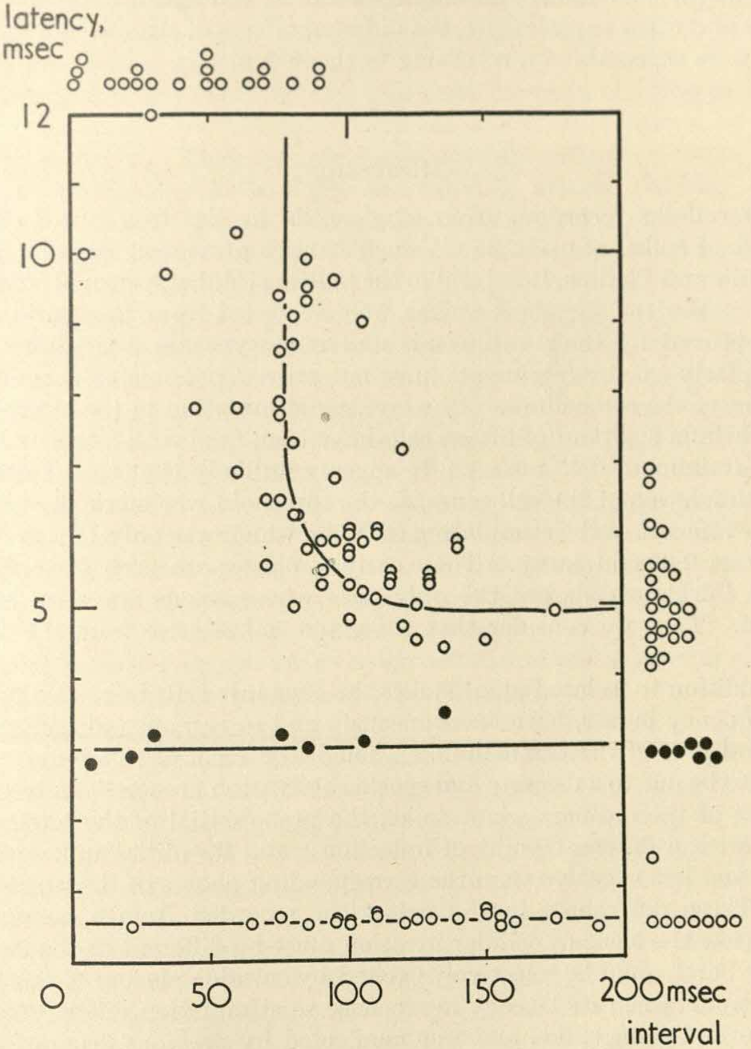

FIG. 11.—Diagram summarizing alternative modes of activation of the cell of figs. 7 and 9. *Abscissa:* interval between shock and preceding natural impulse. *Ordinate:* latency of response. *Open circles:* single impulses. *Filled circles:* bursts. *Right of fig.:* responses in cases in which no natural impulse intervened between successive shocks (interval between shocks was 0·6 sec.). *Top of fig.:* failures to respond to shock. Full explanation in text.

observation. At 0·55 mm., threshold stimulation gave an early impulse, and if the shocks were slightly strengthened this was followed by a later impulse. At 0·675 mm., the threshold was lower, and the response was an early massed wave followed by a late impulse. Slightly stronger shocks added an early impulse. In this experiment no repetitive burst was evoked from any point along the course of this electrode track even by stronger stimulation. In another experiment, stimulation at depth 0·35 mm. elicited an early impulse, and at depth 0·58 mm., and slightly reduced strength, a repetitive burst. In these and other experiments, the different effects of stimulation at different depths were repeatable on returning to those depths.

DISCUSSION

Extracellular recording from single cells in the frog's cerebellum has shown giant spikes of up to 35 mV such as have previously been found in the cat [Granit and Phillips, 1956] and in the bullfrog [Müller, personal communication]. In the cat the giant spikes were recorded from the Purkinje cells, as was proved by their antidromic and monosynaptic activation. In the frog similarly exact experiments have not proved possible on account of the small size of the cerebellum. However, by stimulation in the lateral part of the cerebellum eight out of fifteen cells have been fired with latencies less than 1 msec. (minimum 0·45 msec.). It appears unlikely that this is a result of direct stimulation of the cell soma, as the threshold was markedly altered by small movements of the stimulating cathode, which was only 15 μ in diameter and at least 0·25 mm. away. These early responses are probably antidromic, and the Purkinje cells are the only ones whose axons travel in the right direction. Thus we consider that the giant spikes arise from the Purkinje cells.

In addition to isolated giant spikes the Purkinje cells have also fired brief high-frequency bursts, both spontaneously and in response to stimulation of the lateral part of the cerebellum. Though the form of this burst varies, it appears to be due to a discrete and specific activation process. In comparison with that of the ordinary giant spike, the prepotential of the burst is more gradual with a different point of inflection; and its initial spike goes more positive and less negative than the corresponding phases of the isolated giant spike. These differences in the potentials, recorded from a common site, suggest that the locus of cellular invasion must be different in the two cases. Also, the burst could be selectively excited by suitable placing of the stimulating electrode, when its latency in response to stimulation differed from that of the normal giant spike, and was unaffected by previous firing of the cell. Thus the repetitive response appears to be produced by a specific synaptic mechanism which behaves similarly in spontaneous and in artifically evoked activity.

The special properties and prolonged time course of the burst response may be properties of the afferent system, *e.g.* a stereotyped pattern of granule-cell firing causing a protracted bombardment of the Purkinje cell. Alternatively, the synapses concerned, even when activated by a single afferent volley, may exert a prolonged transmitter action. Repetitive firing in response to single afferent volleys is already familiar in the Renshaw cells of the spinal cord [Eccles, Fatt and Koketsu, 1954], in some of the cells of origin of Flechsig's tract [Laporte, Lundberg and Oscarsson, 1956], and in cells of the cuneate nucleus [Amassian and de Vito, 1954]. Frog Purkinje cells are of special interest in that they can fire either a single impulse or a repetitive burst, and this appears to depend upon which of two discrete afferent systems is thrown into action by a single shock.

The relation, if any, between the repetitive burst in the frog on the one hand, and the inactivation response in the cat on the other, cannot be decided on present evidence. They may be fundamentally different events, or both may be manifestations of a single depolarizing process differing only in intensity. In the cat the inactivation response consisted of an initial diphasic spike followed by a wave lasting 15–30 msec., and accompanied by blocking of the Purkinje cell discharge. In intracellular recording the wave appeared as a persisting depolarization grafted onto the downstroke of the initial spike. Impulse discharge was resumed towards the end of the repolarization, beginning with small spikes which grew to full size as repolarization became complete. In extracellular records the wave was usually positive going, but sometimes negative. Sometimes there were superimposed upon it stunted high frequency spikes [Granit and Phillips, 1956, fig. 6 (3) *b* and *c*]; it was remarked that "this discharge cannot always be seen and apparently requires favourable location of the electrode tip". In a few records [Granit and Phillips, unpublished] such stunted spikes were seen without an underlying wave. The wave and the stunted spikes may be due to a similar depolarizing process, causing repetitive firing at low intensities and "inactivation" at high. Transitional forms of response are to be expected, and were observed in the cat. Quantitative intracellular measurements of magnitude of depolarization in relation to nature of response—repetitive or "inactivating"—are desirable but are probably beyond the reach of present technique.

The probability that all these responses, in frog as well as in cat, are manifestations of a similar underlying process is strengthened by two features of the extracellular records. 1. In frog, as in cat, there is sometimes evidence of persisting depolarization, shown by persisting extracellular negativity of varying degree (figs. 2, 4 and 5). 2. In both cat and frog records, the initial spike of the response is shifted bodily in a positive direction relative to the giant spikes [*cf.* Granit and Phillips, 1956, fig. 6 (1)].

Typical inactivation responses have been seen in one frog only. Their rare occurrence in this single experiment, and the absence of such responses in the remaining experiments, are in contrast with the cat, in which inactivation responses were of very frequent occurrence in every record. In the frog no basket-like ending have been seen [Larsell, 1923; Glees, Pearson and Smith, 1958]. Inactivation in the frog cannot therefore have been due to basket cell activity. The frog result renders redundant, although it does not disprove, the hypothesis that the baskets are the cause of inactivation of Purkinje cells in the cat.

In bullfrog Purkinje cells, Müller and Larremendi [Müller, personal communication] observed prolonged extracellular negative waves, lasting several seconds, after brief tetani to the trigeminal nerve. We have not tetanized this nerve, and have seen no such prolonged events in response to single shocks delivered through a microelectrode in the lateral part of the cerebellum.

ACKNOWLEDGMENT

We wish to thank Mr. F. O'Connor for technical assistance.

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SYNAPSES ON THE PURKINJE CELLS OF THE FROG.

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(*Received for publication* 1*st July* 1957)

A close study of the frog's cerebellum, particularly for the presence of synapses with the Purkinje cells, showed that no such structure as "basket" fibres typical of the mammalian cerebellum can be detected, while fibres resembling climbing fibres are present in abundance. This finding suggests that the main type of synapse is the axo-dendrite type (climbing fibre-Purkinje dendrite). In addition, comparatively few typical ring-like boutons were found in contact with the basal portion of Purkinje dendrites.

The Purkinje cells of the frog are of small size compared with those of mammalian cells and their arrangement and spacing is very irregular. Typical dendrites can clearly be followed only into the first part of the molecular layer, while the larger part of the molecular layer is filled with extremely thin branches of these dendrites, and larger fibres, some of which may be climbing fibres or other afferent fibres. The paper includes general comments on the importance of linking histological and neurophysiological research on the synapse.

INTRODUCTION

STUDIES of synapses, particuarly their degeneration following experimental lesions, have been used for the last fifteen years in tracing pathways to their final termination, either on to the surfaces of cell bodies or on to dendrites. The separation of synapses into axo-somatic and axo-dendritic types for the lateral geniculate body [Glees, 1940, 1941; Glees and Le Gros Clark, 1941; Glees, 1942, 1946] proved of value to the neurophysiological research of Marshall and Talbot [1948], who discussed the physiological significance of these morphological findings on overlap and the mode of termination of optic axons in the lateral geniculate body. The use of microelectrodes for obtaining accurate measurements of increase or decrease in excitability of single neurones has given fresh impetus for further morphological study of synapses. The site of termination of afferent fibres on neurones is of great physiological importance, especially in microelectrode studies when orthodromic and antidromic stimulation is used.

Morphological studies on synapses are of special value if the relevant neurophysiological work can be carried out at the same time. Glees emphasized this to the members of the first Neurobiological Congress [1955], which had as its main topic the study of synapses. For this reason the present investigation was undertaken to integrate to some extent histological appearances with the electro-physiological findings of Matthews, Phillips and Rushworth [1958].

MATERIAL AND METHODS

Twelve frog brains were fixed by immersion in neutral 10 per cent formol-saline and embedded in paraffin wax. Six brains were cut sagittally and the remaining transversely. Serial sections of 3 sagittally and 3 transversely cut brains were stained with silver-nitrate according to the Glees' method modified by Marsland *et al.* [1954]. Two more frog brains were impregnated by the Bielschowsky block impregnation silver method and were embedded in Ester wax [Chesterman and Leach, 1956]. One brain was cut sagittally and 1 was cut transversely. Frozen sections were made from 2 brains and stained with Glees' silver method [1946], and Cajal's cerebellum method. Cresyl violet was used for staining the Nissl granules and in combination with Luxol blue to stain the myelinated fibres and cells together on the paraffin sections [as recommended by Klüver and Barrera, 1953].

The same histological procedures were followed for the cat's cerebellum used for comparison with that of the frog.

Several drawings have been used in this paper. Some histologists prefer photomicrographs to drawings. Synapses, however, can be studied only with objectives of the highest resolution, thus limiting the depth of focus considerably. Wredden [1947] for the oil-immersion lens quotes a depth of focus of no more than 0·5 μ. This implies that in a 10 μ section 20 photographs would have to be taken to obtain a comprehensive record of synaptic connexions, while 1 drawing can combine all these focal planes in one illustration.

HISTOLOGICAL DESCRIPTION

This description is mainly concerned with the Purkinje layer and the problem of whether "baskets" and "climbing" fibres can be found in this layer comparable to those found in the Purkinje layer of mammals; but it also contains some general observations on the frog's cerebellum, as little

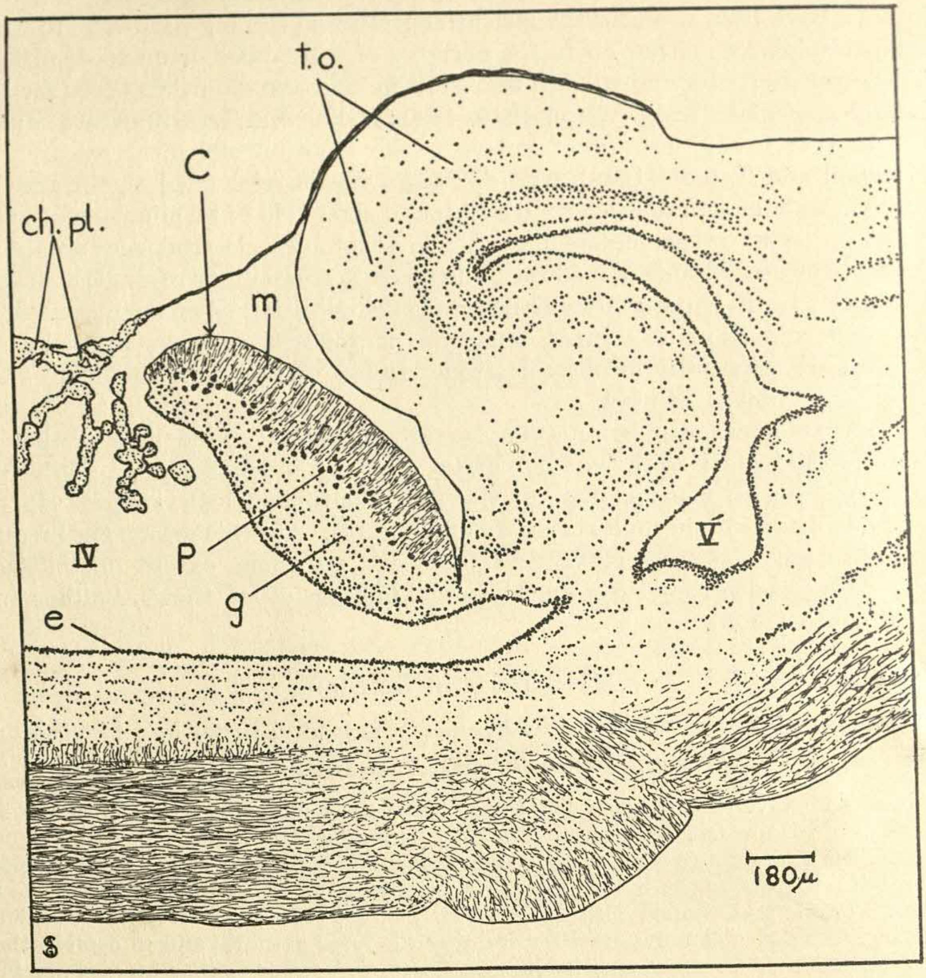

FIG. 1.—Sagittal section through the cerebellum of the frog, stained with silver nitrate. Glees method.

Ch. pl. = Choroid plexus.
IV = 4th ventricle.
C = Cerebellum.
m = molecular layer.
P = Purkinje cell layer.
g = granular layer.
V = Ventricle of the tectum.
t.o. = tectum opticum.
e = ependyma.

information on this is provided even in comprehensive textbooks on neuroanatomy. The general arrangement of the frog's cerebellum in three main layers (comparable to that of mammals) is already clearly visible under low power in the sagittal plane (fig. 1).

It is generally assumed that "basket" and "climbing" fibres represent the important synapses, although ring-like endings (*boutons*) on the stem of the Purkinje dendrites can be observed in the mammalian cerebellum, as described by Glees and Nauta [1955].

The Purkinje layer is characterized by typical flask-like cells, whose curved dendrites can be followed into the first quarter of the molecuar layer (fig. 2). The Purkinje cells of the frog are not regularly spaced or arranged in a single layer (unlike the cat), but are often found "on the top of each other" and grouped into clusters of cells.

ABSENCE OF BASKET FIBRES

Absence of "basket"-like structures is immediately noticeable. There are a few fine fibres in the proximity of the Purkinje cell body, but these fibres do not seem to have that special relationship (either in number or position) to the Purkinje cell body, which can be seen so clearly in the cat (figs. 4*a* and *b*). Furthermore, since shrinkage in sections of the cat's cerebellum does not cause detachment of "baskets" from their related cells, it is unlikely that similar shrinkage in sections of the frog's cerebellum can account for the absence of "basket"-like structures.

DENDRITES AND THEIR SURROUNDING STRUCTURES

The dendrites do not show the strict alignment in the vertical plane, which is so characteristic in the mammalian Purkinje cell layer, and their branches are far less profuse than in the mammalian cerebellum (figs. 2, 3*a* and *b*).

The histological appearance of the stem portion of the dendrites is similar to the cell body when stained with silver methods, namely a light brown coloured cytoplasm in which the darkly staining fibrillæ are embedded; occasionally bouton-like endings in contact with the basal portions of the dendrites are observed, in addition to numerous boutons in the uppermost part of the granular layer (fig. 5). Numerous "climbing" fibres follow the course of these dendrites. This larger type of dendrite, however, is clearly visible only in the lower part of the molecular layer, for the dendrites higher up thin out considerably (as if only their neuro-fibrillæ continued) and are then lost in a plexus of extremely fine interlacing fibres which extend up to the margin of the molecular layer and make it impossible to distinguish whether they are dendrites or afferent fibres. Within this layer lie scattered darkly staining nuclei which may be neuroglia nuclei (fig. 6).

The measurements reported on page 58 are taken from fixed and embedded material, and in both, cat and frog, a shrinkage of approximately 30–35 per cent must be expected. Fig. 3*a* illustrates that in frog cerebellum no "basket"-like endings surround the Purkinje cell body, while "climbing" fibres can be seen. The extreme thinness of the Purkinje cell axon in the frog (0·5–1 μ) is of further interest in contrast to the mammalian Purkinje cell axon which is at least twice that size when emerging from the cell.

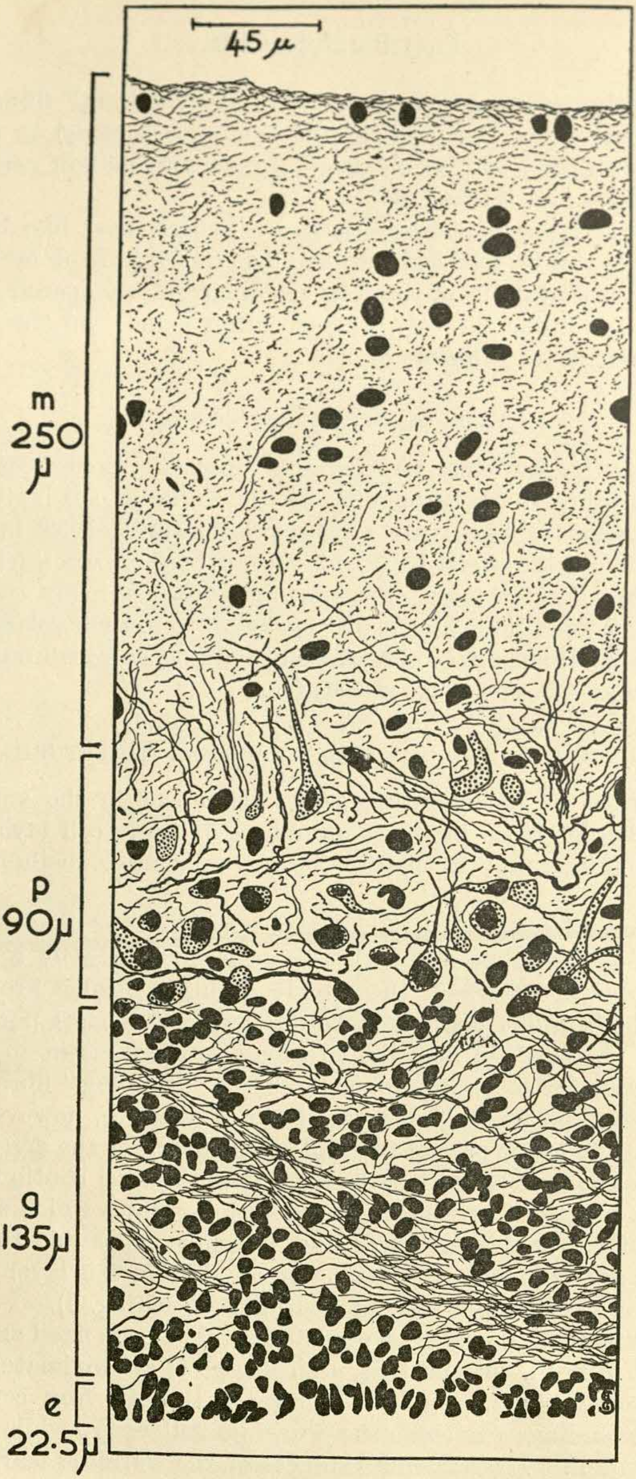

FIG. 2.—Transverse section through the cerebellum of the frog.

m = molecular layer.
P = Purkinje cell layer.
g = granular layer.
e = ependyma.

Table I.—Purkinje Cell Diameters

Measurements of 20 cells in μ

		Cat			Frog		
		Average	Min.	Max.	Average	Min.	Max.
Paraffin embedding							
Nissl cresyl violet	cell	16·4	11	23	11·3	9	15
	nucleus	8·7	5	9	8·4	4	10
Glees silver method	cell	13·9	11	20	9·8	8	15
	nucleus	7·8	5	10	7·3	5	9
Cellosolve ester wax embedding							
Nissl cresyl violet	cell	18.5	17	23	8·7	6	10
	nucleus	8·5	6	11	6·2	5	8
Glees silver method	cell	18·3	14	23	9·4	7	12
	nucleus	6·5	6	10	6·7	5	8

Notes

1. In the Nissl-stained sections of both frog and cat, measurements are taken through a plane showing the nucleolus.

In the silver-stained sections of the frog, embedded in paraffin, measurements are taken through the approximate centre of the cell showing the stem of the Purkinje cell dendrites and the nucleus, as the nucleolus can not be seen. However, in the silver-stained sections embedded in ester wax, measurements are taken in a plane through the nucleolus, as this structure is clearly visible.

2. Comparing these two sets of measurements with special regard to the ratio of the cell body to the nucleus, it is at once apparent that in the frog the nucleus is very large and almost fills the cell body, while in the cat the ratio of cell body and nucleus shows clearly that the nucleus occupies approximately only half the diameter of the cell body.

Comparisons show that the frog's Purkinje cell is about two-thirds the diameter of the cat's (*cf.* figs. 3*b* and 4*b*).

DISCUSSION

When looking for more detailed information about the termination of fibre tracts in lower vertebrates, two significant facts immediately emerge: (*a*) the lack of experimental work available and (*b*) the almost exclusive use of the Golgi technique in such work as exists. These criticisms should not, of course, belittle the great work of Herrick [1948] on the salamander; but many pathways will have to be verified by experimental methods, as the study of serial sections of the normal brain stained with Weigert's or Golgi's technique does not reveal the precise localization of fibre tracts or their terminations. The Golgi technique can be deceptive, for the incomplete staining of this method is apt to be overlooked and its results are too liberally interpreted to be of general significance. It is this technique particularly which gives rise to the diagrammatic plotting of brain connexions, thus suiting a prevailing view in neurophysiological thinking, but ultimately hindering scientific progress. The histological study of the frog's cerebellum by Larsell [1923] is inadequate for the above reasons. Here silver methods can be used more advantageously. Judging from our material, we can state that the frog's Purkinje cell body is not surrounded by a densely interwoven plexus of

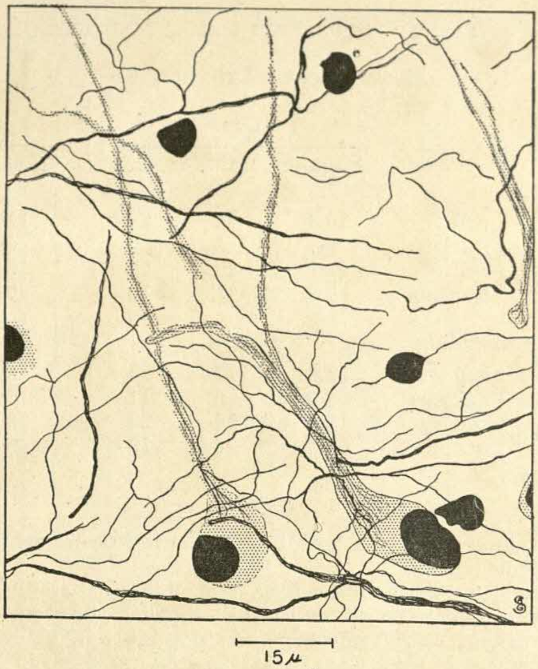

FIG. 3a

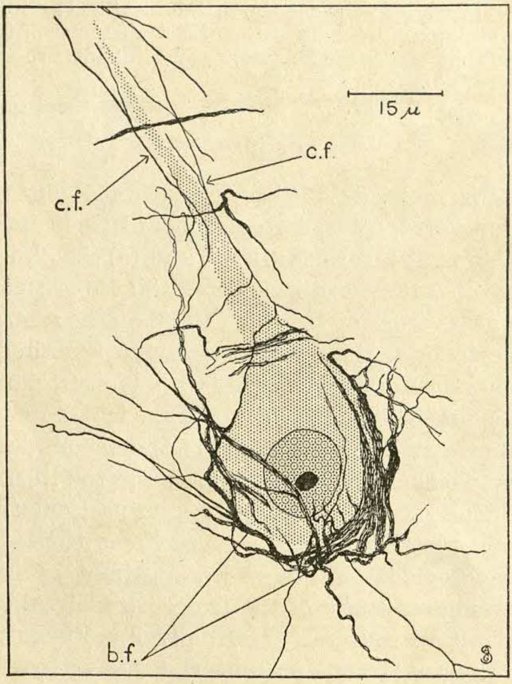

FIG. 4a

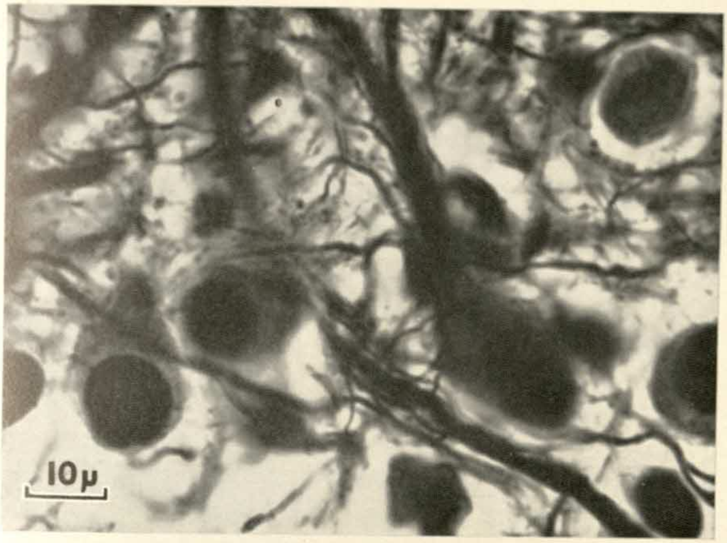

FIG. 3b.—Two Purkinje cells of the frog under oil immersion. Note the large nucleus in each Purkinje cell. The scattered black nuclei belong to cells of the molecular layer. The dendrites of the Purkinje cells are surrounded by fine fibres, but the cell bodies are devoid of basket fibres.

In fig. 3a the two cells have been drawn; in fig. 3b the same cells have been photographed.

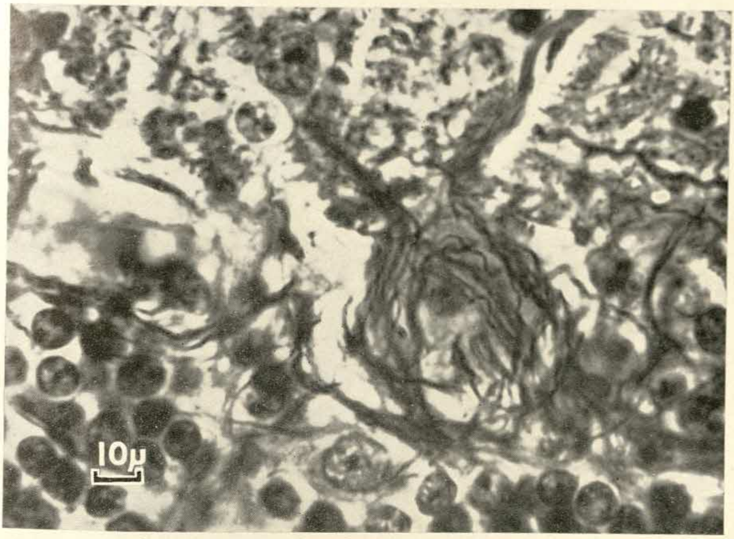

FIG. 4b.—A large Purkinje cell of the cat's cerebellum under oil immersion, showing the basket fibres (b.f.) and the climbing fibres (c.f.). Compare with figs. 4a and b.

In fig. 4a the cell has been drawn; in fig. 4b the cell has been photographed.

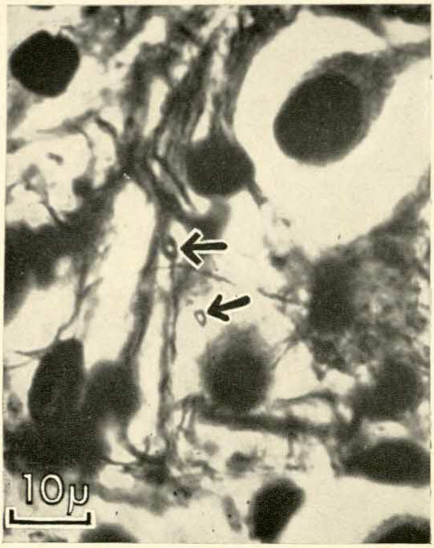

FIG. 5.

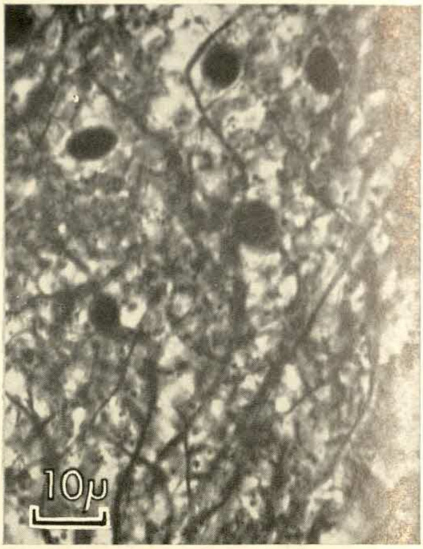

FIG. 6.

FIG. 5.—The presence of boutons can be shown in the upper granular layer. (Oil immersion, photomicrograph.) Frog.

FIG. 6.—The upper molecular layer of the frog's cerebellum. Note intermingling of climbing fibres and fine dendrites of Purkinje cells. (Oil immersion, photomicrograph.)

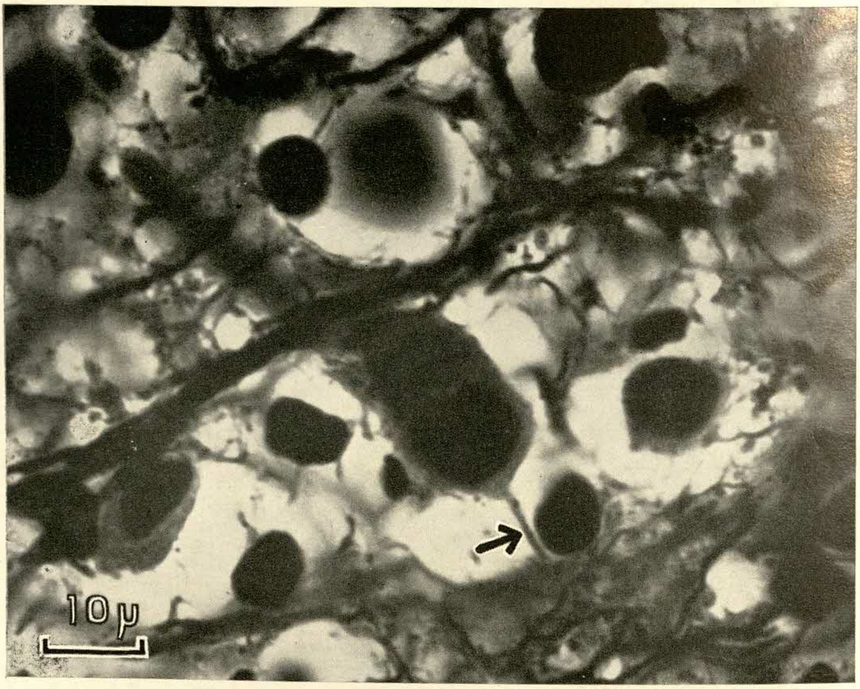

FIG. 7.—A photomicrograph under oil immersion of a Purkinje cell of a frog to illustrate the extreme thinness of the emerging axon, marked by arrow.

fibres referred to as a "basket", which shows up clearly when using the same technique on the cat (compare figs. 3*a*, *b* with figs. 4*a*, *b*). Furthermore, as the finest fibres, including the intracellular fibrillæ, have been impregnated, we have no doubt that the "basket" fibres are absent in the frog's cerebellum. The axons of the Purkinje cells after emerging are extremely thin protoplasmic strands and can be followed for a distance of about 10 μ before they are lost among the fibres of the granular layer (fig. 7).

Possibly synapses may be established between the "climbing" fibres and Purkinje dendrites of the frog, but apart from the intimate contact between these two structures, no definite histological basis for synaptic contact can be detected. The "climbing" fibres do not enlarge at the point of close contact, and only electron-microscopical studies (revealing mitochondria or special membranes) will be able to decide whether the significance of the "climbing" fibres lies in synaptic transmissions as is suggested by classical teaching.

There is a wide field for electron-microscopical studies in dealing with these problems. Palade and Palay [1954], Young [1956], and Palay [1956] clearly demonstrate the value of this new method in a field where silver methods have reached their limit.

Our search for more definite structures for synaptic transmission on the Purkinje cells of the frog produced in the main a negative answer; as there are no "basket"-like endings round the cell soma. Comparatively few boutons have been found in contact with the stem dendrites of Purkinje cells, and their origin could not be ascertained. "Climbing" fibres do exist, but their rôle as synaptic structures is based only on assumptions.

While treating this assumption cautiously, it is nevertheless reasonable to argue that "climbing" fibres in contact with dendrites are the main structures present which could be endowed with synaptic properties, apart form the boutons described above. Should this assumption prove to be true, it would follow that the Purkinje cell of the frog has only axo-dendritic types of synapses. It could therefore be asked: can the upper portions of the molecular layer have synaptic connexions of a more diffuse nature? For it is this layer in which dendrites and climbing fibres "embedded" in a granular ground substance are in close contact with each other.

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THE UPTAKE OF SULPHUR-35 BY THE TRACHEA AND GALL-BLADDER. By M. A. JENNINGS. From the Sir William Dunn School of Pathology, Oxford.

(*Recieved for publication* 23*rd August* 1957)

The mucous cells in the glands and surface epithelium of the trachea of the cat, rabbit, guinea-pig and rat took up ^{35}S from injected $Na_2{}^{35}SO_4$ and incorporated it in their mucin. In the mouse uptake was very slight, most of the mucous cells remaining free of ^{35}S. The mucous cells which line the gall-bladder and larger bile ducts of the cat took up ^{35}S and incorporated it in their mucin. The few mucous cells of the rabbit biliary tract also appeared to do so. As has previously been shown, the cartilage cells of the trachea strongly take up ^{35}S; as far as autoradiographs could show the timing of uptake and the distribution of the ^{35}S in cartilage were identical in the five species examined.

THE incorporation of sulphate into the epithelial mucus of the gastro-intestinal canal was studied by identifying in autoradiographs the sites where ^{35}S, injected as $Na_2{}^{35}SO_4$, was localized [Jennings and Florey, 1956]. The localization of ^{35}S in two other mucus-forming epithelia, those of the trachea and gall-bladder, is recorded here. The distribution seen in the tracheal cartilage, which confirms other observations, is also recorded.

EXPERIMENTAL

Tissues from animals injected with $Na_2{}^{35}SO_4$ were fixed, sectioned, and stained by the periodic acid-Schiff (P.A.S.) reaction to show up mucin. They were then covered by stripping film [Doniach and Pelc, 1950] and exposed for from 2 to 12 weeks. In the resulting autoradiograph silver particles in the emulsion could be related to the structures in the section that had been stained by the P.A.S. reaction. The sections were sufficiently washed during preparation for free sulphate to be removed; any radioactivity remaining in the section was considered to be due to ^{35}S in some way "incorporated" in the tissue. In interpreting the autoradiographs comparison was made with ordinary sections from the same blocks stained by the P.A.S. reaction and by mucicarmine. The technique used for preparation of the material was described in detail by Jennings and Florey [1956] and by Heatley, Jerrome, Jennings and Florey [1956].

The following animals were used, the interval between the injection of $Na_2{}^{35}SO_4$ and killing being shown in parenthesis:

Trachea.—Two cats (2¼ and 6 hr.); 3 rabbits (2¼, 6½ and 24 hr.); 2 guinea-pigs (2 and 6½ hr.); 4 rats (2 at 2 hr. and 2 at 24 hr.); 4 mice (3, 6, 12 and 24 hr.).

Gall-bladder.—Two cats (2¼ and 6 hr.); 2 rabbits (2¼ and 24 hr.); 4 mice (3, 6, 12 and 24 hr.).

The weights of the animals were: cats, 1·4 and 1·6 kg.; rabbits, 1·5 kg.; guinea-pigs, 200 g.; rats, 150–200 g.; mice, 24–26 g.

RESULTS

Trachea

Surface Epithelium.—In the cat, rabbit, guinea-pig and rat the goblet cells of the surface epithelium contained radioactive material. In the cat and rabbit at 2¼ hr. after injection the radioactivity was largely concentrated in the supranuclear region of each cell, but in many of the goblet cells of the guinea-pig and rat it was at this time in the main intracellular mucin mass. At 6 hr. the picture was much the same (rabbit and guinea-pig *) but at 24 hr. only a trace of activity was left in the superficial parts of some cells (rabbit and rat).

In the rats killed 2 hr. after injection there was a continuous layer of radioactivity in the border of all the epithelial cells, not corresponding with any structure staining for mucin either by the P.A.S. reaction or by mucicarmine. This layer was distinct from the free mucus lying on the surface of the epithelium.

In the mouse the occasional goblet cell of the surface epithelium contained no radioactivity at any time.

Glands.—The submucosa in the cat contained plentiful mucous glands. At 2¼ hr. after injection there was strong activity in the intracellular mucin masses and in free material in the gland lumina. At 6 hr. activity was more markedly concentrated towards the free borders of the cells and in the gland lumina.

The mucosa of the guinea-pigs contained a few mucous glands which were strongly radioactive, and those of the rabbit and rat an occasional mucous acinus showing activity; in these the intracellular location of the activity could not be identified.

In the mouse there were many mucous glands but only an occasional acinus showed a trace of activity.

Free Mucus on the Surface.—When present, this was radioactive in all specimens taken 2–6 hr. after injection, with the exception of those from the mouse. In the rat and guinea-pig, the mucus was apparently coming from the goblet cells, as well as presumably from the few mucous acini. In the cat killed 2¼ hr. after injection the abundant mucous glands appeared to be contributing largely to the free mucus on the surface since radioactive mucus was present in the gland lumina but in most of the goblets had not yet reached the free end of the cell. In the cat killed at 6 hr. the glands were apparently the sole source of the free mucus, as surface epithelium was metaplastic and contained no goblet cells, though it is possible that in neighbouring parts of the trachea the surface epithelium was more normal.

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* The surface epithelium of the cat killed 6 hr. after injection was metaplastic as a result of infection and contained no goblet cells.

In the mouse the surface mucus was not radioactive.

Gall-bladder

Of the three species from which the gall-bladder with a portion of liver was taken for section, only the cat had the gall-bladder lined by mucous cells. In the cat all the columnar cells contained some mucus droplets, gathered in the supranuclear region or forming a column from there to the surface. These cells showed radioactivity. In the cat killed 2¼ hr. after injection the activity was concentrated in the supranuclear region; in that killed 6 hr. after injection the activity was distributed through the intracellular mucin and was present also in free mucus in the crypts.

In the rabbit and mouse a few epithelial cells showed a trace of staining for mucin at their free borders. In the rabbit killed 2¼ hr. after injection these areas were faintly radioactive, in the mouse they showed no activity.

In the rabbit killed at 24 hr. there was a continuous layer of faint radioactivity in the cell borders, although there was no corresponding staining for mucin.

Bile Ducts

In the bile ducts of the cat the epithelium showed radioactivity where it contained mucus, that is to say in the largest ducts. The bile duct epithelium of the rabbit contained scattered goblet cells, and the mucus in these showed faint activity.

Liver

In the cat killed at 2¼ hr. after injection, and to a lesser extent in that killed at 6 hr., there were in the liver localized concentrations of radioactivity whose size and distribution suggested that they might be connected with the Kupffer cells, though they could not be localized exactly in these sections. The other two species showed no sign of this type of distribution.

Cartilage

All the five species showed similar uptake of radioactivity. Two or 2¼ hr. after injection there was definite concentration of radioactivity in the cartilage cells and apparently also in the unstained area round each cell. At 24 hr. after injection the amount of radioactivity appeared to be greater, the distribution being precisely the same.

It did not appear that even at 24 hr. any radioactivity had entered the matrix, but owing to scatter of the silver particles it was difficult to be sure; the amount if any must have been small.

DISCUSSION

The previous paper [Jennings and Florey, 1956] showed that the various types of mucous cell in the stomach and intestine varied greatly in the extent to which they incorporated ^{35}S from injected $Na^{35}SO_4$ into their secretion. There were also differences in this respect between species. For this reason it has seemed worth while to record the findings in two other epithelial mucus-producing tissues.

Not much has previously been reported about the uptake by the respiratory tree of ^{35}S from injected sulphate. Rat and rabbit tracheal mucosa and respiratory epithelium of the lung showed in autoradiographs a moderate degree of uptake of ^{35}S [Odeblad and Boström, 1953; Boström, 1953]. Though the cells concerned were not identified Boström [1953] thought it "possibly due to incorporation in the mucus". The present work showed that in the rat, rabbit, cat and guinea-pig both the goblet cells and the cells of the mucous glands of the trachea take up ^{35}S. Curran and Kennedy [1955] found no uptake of ^{35}S in the respiratory epithelium of new-born mice injected with $Na_2{}^{35}SO_4$ although the epithelium stained with alcian blue, and this accords with the present finding that in the trachea of the mouse the goblet cells took up no ^{35}S and the mucous glands showed only an occasional trace of radioactivity. Like the cells of the gastro-intestinal tract, the mucous cells of the trachea vary in the rate at which they take up ^{35}S into their secretion. This was seen best in the cat in which 2¼ hr. after injection the goblet cells had ^{35}S concentrated in the supranuclear region, the "goblet" or main mucin mass being generally still free of activity; but at this time most of the gland cells contained a radioactive mucin mass and were passing radioactive mucin into the gland lumina. This variation, however, was trivial compared to the regularity with which, in all the species examined, those epithelial cells which took up ^{35}S did so in the first hours after injection and largely discharged it by 24 hr.

The gall-bladder does not seem to have been examined before, but Curran and Kennedy found a little uptake of ^{35}S, corresponding with a little alcian blue staining, in the bile duct epithelium of new-born mice. In the mice used in the present work there was only a trace of staining by mucicarmine and the periodic acid-Schiff reaction in the gall-bladder epithelium, and no uptake of ^{35}S. In the rabbit similar traces of staining for mucin corresponded with areas of faint radioactivity and some goblet cells which were present in the bile ducts took up ^{35}S. The cat differs from these two species in having the gall-bladder and larger bile ducts lined by an epithelium all the cells of which secrete mucus [Harding, 1934], and these cells strongly took up ^{35}S. Although they are morphologically somewhat like the cells lining the stomach, their secretion is different, since neither in the cat nor any other species examined did the gastric lining cells take up ^{35}S from injected $Na_2{}^{35}SO_4$ [Jennings and Florey, 1956].

There is nothing new in the observations made on the tracheal cartilage except that in the five species used, so far as autoradiographs could show, the rate and pattern of uptake was the same. Cartilage has been extensively used for study of the formation and isolation of chondroitin sulphate labelled by ^{35}S [for instance Dziewiatkowski, 1951; Boström, 1952; both on the rat]. Tracheal cartilage has been shown, like other cartilages, to take up ^{35}S from injected $Na_2{}^{35}SO_4$ [Odeblad and Boström, 1952; Boström and

Odeblad, 1953; both by autoradiography, rat and rabbit]. That the uptake of ^{35}S is dependent on the activity of the cartilage cells has been demonstrated [Boström and Månsson, 1952, 1953–54, both on calf; Dziewiatkowski, 1954, rat; Curran and Gibson, 1956, man] and in autoradiographs the radioactivity has been localized in and around the cartilage cells [Davies and Young, 1954]. Curran and Gibson [1956] from studying autoradiographs of human cartilage slices believed that radioactivity was located solely in the cells, and was less in younger and in vacuolated than in mature cells.

The strong uptake of ^{35}S by cartilage cells has been used as a test of the survival of cartilage in experimental grafting [Bacsich and Wyburn, 1955, guinea-pig] and in the storage of cartilage grafts [Gibson, Curran and Kennedy, 1955, man]. It was perhaps because their mice were newly born that Curran and Kennedy [1955] found a good deal of radioactivity in the matrix as well as in the cells of cartilage since, relative to other tissues, the uptake of sulphate by cartilage is immensely greater in the fœtus than in the adult [Dziewiatkowski, 1953, rat], and the uptake diminishes with increasing age [Boström and Månsson, 1953–54, cow]. In young rats Duthie and Barker [1955] found ^{35}S activity in both cells and matrix of articular cartilage, and uptake was most active in regions of greatest growth. It is of interest that in recent work the only polysaccharides identified in cartilage were sulphated compounds [Meyer, Davidson, Linker and Hoffman, 1956].

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THE OXIDATION OF ^{14}C LABELLED CHYLOMICRON FAT BY THE RAT.

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The oxidation of ^{14}C labelled tripalmitin incorporated into homologous chyle has been studied in rats. Amounts of fatty acid varying from 10 to 90 mg. were injected intravenously into rats starved for 0–72 hr. Following the injection of the labelled chyle the output of $^{14}CO_2$ and the specific activity of the expired CO_2 rose rapidly and reached a maximum in 1–2 hr. The rate of oxidation of the tripalmitin was increased by starvation and the percentage oxidized was inversely related to the amount of fatty acid injected. In non-starved rats injected with 5–10 mg. of fatty acid approximately 1·9 per cent was oxidized per mM of expired CO_2 in the first 2 hr. following the injection. In animals starved for 72 hr. the rate of oxidation of the fat was increased to approximately 3·5 per cent per mM of CO_2 expired during the first 2 hr. The feeding of glucose or olive oil in isocaloric amounts to rats starved for 24 hr. had a similar effect on the oxidation of injected ^{14}C labelled tripalmitin reducing the amount oxidized to CO_2 by about 14 per cent. Similarly, the intravenous injection of glucose or homologous unlabelled fatty chyle in isocaloric amounts caused an equal reduction in the rate of oxidation of injected labelled tripalmitin. There was no evidence that the rat oxidized glucose preferentially when both chylomicron fat and glucose were available for metabolism.

It has been shown conclusively in several species of animals that long chain fatty acids are metabolized when injected intravenously in the form of artificial emulsions [McKibbon, Ferry and Stare, 1946; Geyer, Chipman and Stare, 1948; Lerner, Chaikoff, Entenman and Dauben, 1949 *a* and *b*]. The rate of oxidation is influenced by the nutritional state of the animal and significant differences occur in animals which have been fasted or fed carbohydrate before the administration of the fat emulsions [Geyer and Waddell, 1951; Geyer, Bowie and Bates, 1953; Lossow and Chaikoff, 1955]. Despite much work on the biochemical nature of the oxidation of long chain fatty acids, there is little quantitative information about the rate at which these acids are oxidized under physiological conditions.

To study this problem, ^{14}C labelled tripalmitin has been incorporated into homologous rat chylomicra. This labelled chyle has been injected intravenously into rats to establish the rate at which the palmitic acid molecules are oxidized to CO_2 under different physiological conditions and to establish some of the factors which normally influence the rate of metabolism of this fat.

METHODS

Collection of Labelled Chyle.—^{14}C labelled chylomicra were obtained from rats in which thoracic duct fistulæ had been established previously by the technique of Bollman, Cain and Grindlay [1948]. Through a stomach tube, 30 μc. of glyceryl-l-^{14}C-tripalmitate were given dissolved in 1 ml. of warm olive oil and the radioactive chyle collected into citrate during the subsequent period of fat absorption. The radioactive content of this chyle was present in the chylomicra presumably as tripalmitin [French and Morris, 1957].

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Injection of Chyle and Collection of Expired CO_2.—Albino rats of both sexes weighing 100 ± 5 g. were fed a stock diet of rat nuts (56 per cent carbohydrate, 3·9 per cent fat, 16·9 per cent protein) and provided with water *ad libitum* up to the time of the experiment. These animals were used as the non-starved subjects or were deprived of food, but not water, for periods up to 72 hr. for the starvation experiments. The labelled chyle was injected intravenously in volumes containing 10–90 mg. of total fatty acid. Injections were given through an exposed femoral vein under light ether anæsthesia. The period of injection and recovery lasted 2–3 min. The rats were then placed in a metabolism cage and provided with water, but no food, throughout the experiment. The cage was connected to an absorption line through which CO_2 free room air was drawn at a rate of approximately 5 l./min. The air leaving the chamber was dispersed through a sintered glass disc into a tower containing 100 ml. of initially carbonate free N/l. sodium hydroxide solution. The CO_2 expired was collected into this solution which was replaced at hourly intervals during the 8 hr. after injection of the radioactive chyle. Towers containing saturated solutions of barium hydroxide were used to check the completeness of CO_2 absorption on both sides of the metabolism chamber.

Radioactivity Assay.—Duplicate 1 ml. aliquots of the sodium hydroxide solutions were taken and the carbonate precipitated with an excess of barium chloride solution in a specially constructed glass filtration cone. The sodium hydroxide solution was injected into the barium chloride through a small hypodermic needle to give a fine dispersion of the precipitate and the fluid allowed to filter through a tared 2·4 cm. disc of Whatman No. 1 filter paper under a stream of nitrogen. The precipitate was collected and washed successively with CO_2 free water, 1:1 acetone water mixture and acetone and then dried under an infra-red lamp. The filter-paper disc with the precipitate was then removed from the filtration apparatus and placed in a brass holder to keep the preparation flat. After drying over phosphorus pentoxide, the disc was weighed and the radioactivity of the sample was counted to an error of ±5 per cent, with a thin mica end-window G.M. tube. All activities were corrected to zero mass. The radioactivity and weights of the duplicate barium carbonate samples agreed within ±5 per cent.

The samples of chyle were oxidized to CO_2 using the Van Slyke–Folch wet oxidation procedure [Van Slyke and Folch, 1940]. Sixty-six μl. samples were oxidized with 5 ml. of perchloric acid oxidant and the CO_2 collected in 50 ml. of initially CO_2 free N/l. sodium hydroxide. Aliquots of this sodium hydroxide solution were then taken and treated as described above.

Total esterified fatty acids were measured by the colorimetric method of Stern and Shapiro [1953].

RESULTS

The Effect of Starvation and of the Amount of Fat injected on the Rate of Oxidation.—To estimate the rate at which the ^{14}C tripalmitin was oxidized an experiment was carried out in which groups of rats were starved for periods from 0 to 72 hr. and injected with amounts of chyle containing from 10 to 90 mg. of fatty acids. The expired CO_2 was collected for 2 hr. following the chyle injection and the percentage of the injected labelled fat which was converted to CO_2 was measured. Fig. 1 shows the relationship of the amount of fatty acid injected and the period of starvation to the percentage of the

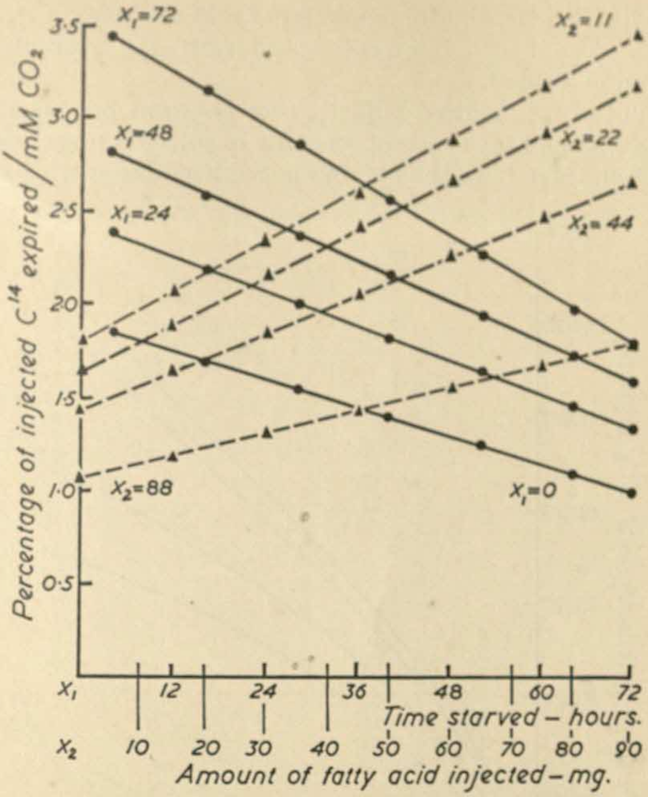

Fig. 1.—The oxidation of ^{14}C labelled tripalmitin by the rat. The graph shows the percentage of the ^{14}C expired per mM of CO_2 produced during the 2 hr. period following the injection of ^{14}C labelled tripalmitin incorporated into homologous chyle.

Table I.—The Relationship of the Period of Starvation in Days (X_1) and of the Amount of Fatty Acid injected in mg. (X_2) to the Percentage of the Injected ^{14}C Tripalmitin expired per mM of CO_2 (Y) during a 2 hr. Period following the Intravenous Injection into Rats of Homologous Fatty Chyle containing Glyceryl-I-^{14}C Tripalmitate.

Regression equation

$$Y = 1{\cdot}877 + 0{\cdot}56111X_1 - 0{\cdot}00957X_2 - 0{\cdot}00346X_1.X_2$$

1st Regression coefficient

$b_1 = 0{\cdot}56111 \pm 0{\cdot}06750 \quad P = < 0{\cdot}001$

2nd Regression coefficient

$b_2 = -0{\cdot}00957 \pm 0{\cdot}00249 \quad P = < 0{\cdot}001$

3rd Regression coefficient

$b_3 = -0{\cdot}00346 \pm 0{\cdot}00133 \quad P = 0{\cdot}01$

injected ^{14}C labelled tripalmitin oxidized per mM of expired CO_2 during this 2 hr. period. The figure represents graphically the multiple regression equation given in Table I.

The amount of fat oxidized is plotted for constant periods of starvation along the lines X_1 and for constant amounts of injected fatty acid along the lines X_2. From these lines the percentage oxidized per mM of expired CO_2 can be read off for rats starved from 0 to 72 hr. and injected with amounts of fatty acid up to 90 mg. The interaction effect between the amount of fatty acid injected and the period of starvation was significant and consequently the lines are not parallel.

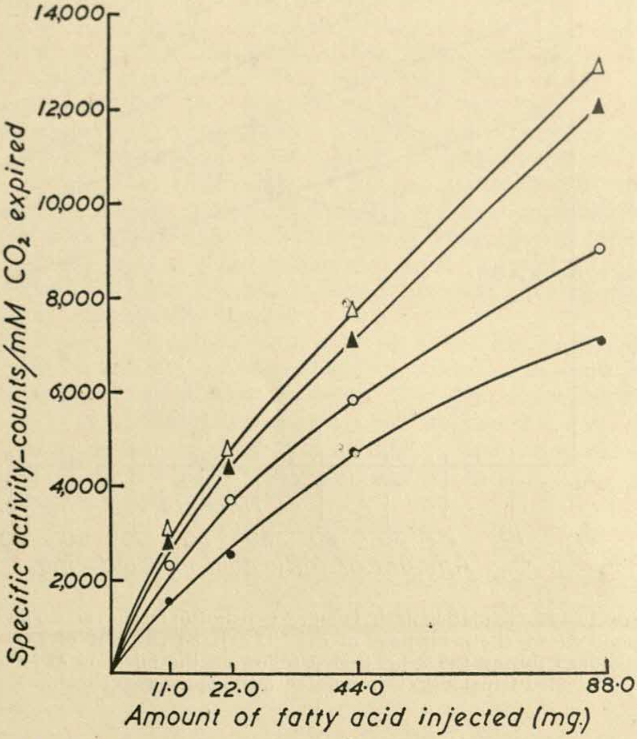

Fig. 2.—The specific activity of the expired CO_2 of rats starved for different periods of time and injected with different amounts of chyle containing ^{14}C labelled tripalmitin. Each point represents the mean of two experiments.

●——● : non-starved.
○——○ : starved 24 hr.
▲——▲ : starved 48 hr.
△——△ : starved 72 hr.

In the groups of starved rats there was a significant reduction in the amount of CO_2 expired. Non-starved rats produced an average of 7·8 mM of CO_2 per hour whilst those starved for 24, 48 and 72 hr. expired on the average 10·3, 19·2 and 23·1 per cent less CO_2 respectively. Fig. 1 shows that rats fed on the stock laboratory diet and receiving 5–10 mg. of fatty acid intravenously would oxidize about 1·7 per cent of this per mM of CO_2 expired during the 2 hr. period following its entry into the blood stream. This represents about 27 per cent of the fat injected. The injection of 5–10 mg. of fatty acid would be equivalent to the amount of fat entering the circulation from the thoracic duct in about 10 min. during the absorption of a fatty meal. In animals which had been starved, the rate of oxidation was increased and after 72 hr. starvation, following the injection of 5–10 mg. of total fatty acid, about 3·5 per cent was oxidized per mM of CO_2 expired. This was about 42 per cent of the injected tripalmitin. When increasing amounts of fatty acid were injected, the percentage oxidized to CO_2 fell off and in non-starved rats about 1 per cent was oxidized per mM of CO_2 expired following the injection of 90 mg. of fatty acid (16 per cent in 2 hr.) and about 1·8 per cent per mM of CO_2 expired in rats starved for 72 hr. (22 per cent in 2 hr.).

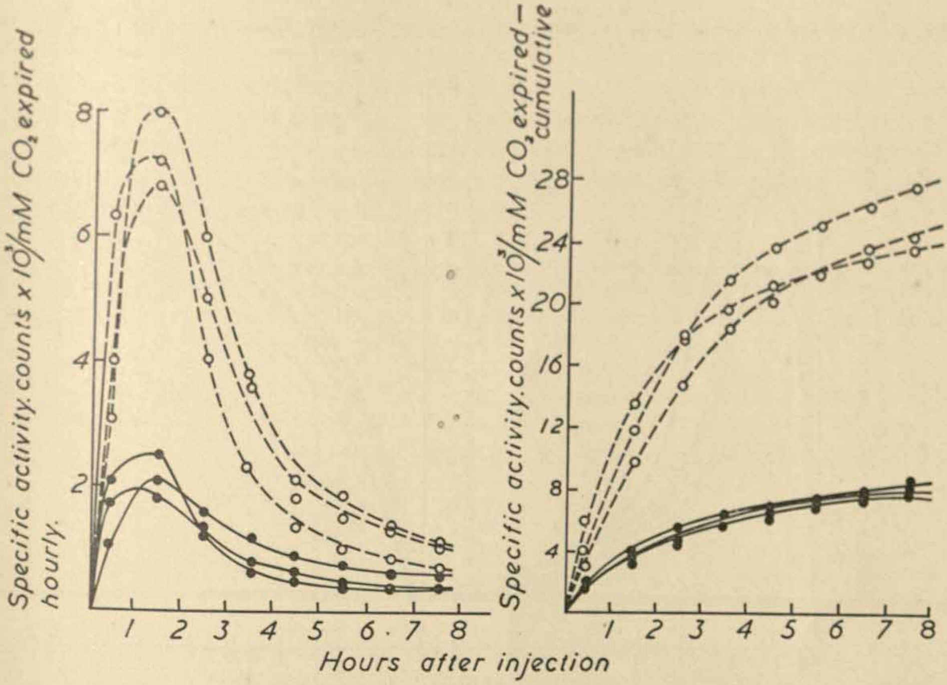

Fig. 3.—The specific activity-time relationships of the expired CO_2 for two groups of rats injected with ^{14}C labelled tripalmitin. 50 mg. of total fatty acids were injected in each experiment and the results are shown for the individual animals.

●———● : non-starved.

○-----○ : starved 24 hr.

As the starved rats had higher absolute rates of fatty acid oxidation and produced less CO_2, the specific activity of the expired CO_2 was much higher than in the non-starved animals. Fig. 2 shows the mean specific activities of the expired CO_2 in four groups of rats during the 2 hr. period following the injection of amounts of fatty acid from 11 to 88 mg. In the non-starved animals the rate of increase in specific activity of the CO_2 with increasing amounts of injected fat was very much slower than in the starved rats. After the injection of 88 mg. of fatty acid, in the non-starved rats there was an appreciable fall off in the increase in specific activity of the CO_2 whereas in rats starved for 72 hr. the CO_2 specific activity increased almost linearly up to an injected dose of 88 mg.

The specific activity-time relationships of the expired CO_2 following the injection of 50 mg. of total fatty acid into starved and non-starved rats are shown

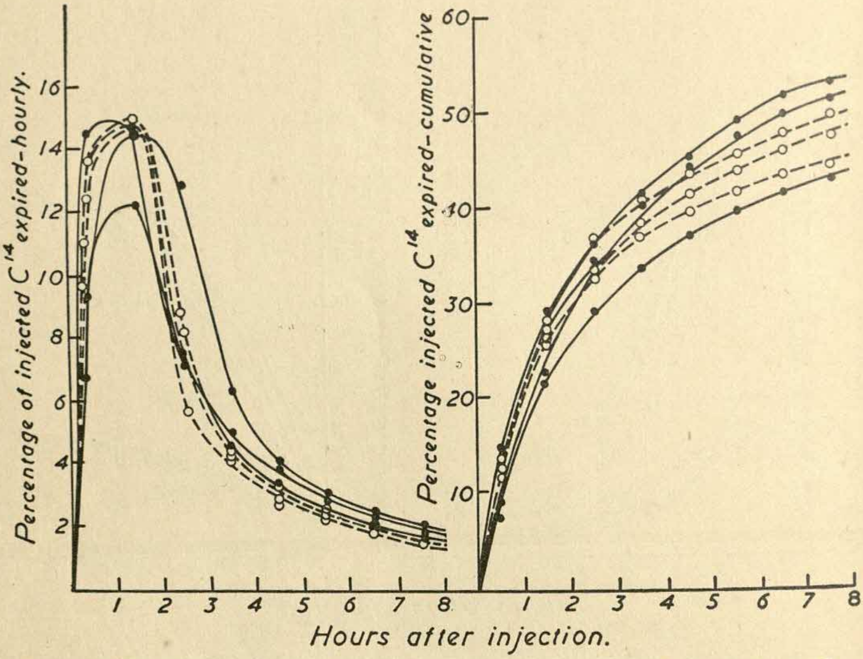

FIG. 4.—The effect of the intravenous injection of glucose or fatty chyle on the oxidation of ^{14}C labelled tripalmitin. The percentage of the injected ^{14}C tripalmitin oxidized to CO_2 is shown hourly and cumulatively over an 8 hr. period following the injection of 50 mg. of chyle fat into rats. The results for the individual animals in each group are given.

○———○ : injected 145 mg. of glucose.
●———● : injected 64 mg. of chyle fatty acids.

in fig. 3. The output of $^{14}CO_2$ rose rapidly and the peak production and specific activity occurred in both groups between 1 and 2 hr. after the injection and then fell off exponentially over the 8 hr. experimental period. In the starved animals the specific activity of the CO_2 reached a level 3–4 times that of the non-starved group within the first 2 hr. and remained significantly higher throughout the 8 hr. period following the chyle injection.

The Effect of giving Fat or Glucose Intravenously or by Mouth on the Oxidation of Intravenously Injected ^{14}C Tripalmitin.—In view of the differences in oxidation rate found between starved and non-starved animals, the effect of simultaneous administration of carbohydrate or fat on the rate of oxidation of ^{14}C tripalmitin was investigated. In the first experiment, two groups of rats which had been starved previously for 24 hr. were used. One group was given fat intravenously in the form of homologous unlabelled fatty chyle, 30 min. before the injection of chylomicra labelled with ^{14}C tripalmitin. A second group was given an approximately isocaloric amount of glucose. Half the glucose solution was given intravenously and half intraperitoneally 15 min. before the injection of the labelled chyle fat. The results are shown in fig. 4. The animals receiving the injections of unlabelled chyle produced an average of 9 per cent less CO_2 per hour than the rats injected with glucose but there was no significant difference between the two groups in the percentage of ^{14}C expired over the 8 hr. experimental period. The injections of these amounts of fat or carbohydrate reduced the amount of ^{14}C tripalmitin oxidized to CO_2 in 8 hr. in both groups by about 14 per cent below that oxidized by rats starved for 24 hr. (Table II).

TABLE II.—THE OXIDATION OF ^{14}C LABELLED TRIPALMITIN BY THE RAT. VALUES GIVEN SHOW THE PERCENTAGE OF THE INJECTED ^{14}C EXPIRED AS CO_2 DURING AN 8 HR. PERIOD FOLLOWING THE INTRAVENOUS INJECTION OF RADIOACTIVE HOMOLOGOUS CHYLE CONTAINING 50 MG. OF TOTAL FATTY ACIDS.

Non-starved	Starved 24 hr.	Injected Glucose (145 mg.)	Injected Fat (64 mg.	Fed Glucose * (1125 mg.)	Fed glucose † (1125 mg.)	Fed Fat (500 mg.)
40·7	63·7	44·4	42·8	41·6	49·0	48·1
31·9	56·6	47·5	53·2	39·6	44·2	48·3
32·9	62·2	49·3	51·9	40·4	43·6	45·2
48·3	64·9	..	..	..	..	..
Mean 38·5	61·8	47·1	49·3	40·5	45·6	47·2

* Fed 30 min. before injection of the labelled chyle.

† Fed 4 hr. before injection of the labelled chyle.

Similar experiments were carried out giving either glucose or fat by mouth to rats previously starved for 24 hr. Those fed with fat were given 500 mg. of olive oil by stomach tube 4 hr. before the injection of 50 mg. of labelled chyle fat. Those fed carbohydrate were given 1125 mg. of glucose by mouth 4 hr. before the chyle injection. Fig. 5 shows the results of these experiments. Again there was no significant difference between the two groups in the amount of injected ^{14}C tripalmitin expired in the 8 hr. period following the chyle injection. The animals fed fat produced on the average 6 per cent less total CO_2 per hour than the carbohydrate fed rats. The percentage of ^{14}C tripalmitin oxidized in 8 hr. by both groups was about 15 per cent less than in rats starved for 24 hr. (Table II).

Three additional experiments were carried out on starved rats in which the period between the feeding of glucose and the injection of labelled chyle was reduced. 1125 mg. of glucose were given by mouth 30 min. before injecting 50 mg. of the labelled chyle intravenously. In these animals about

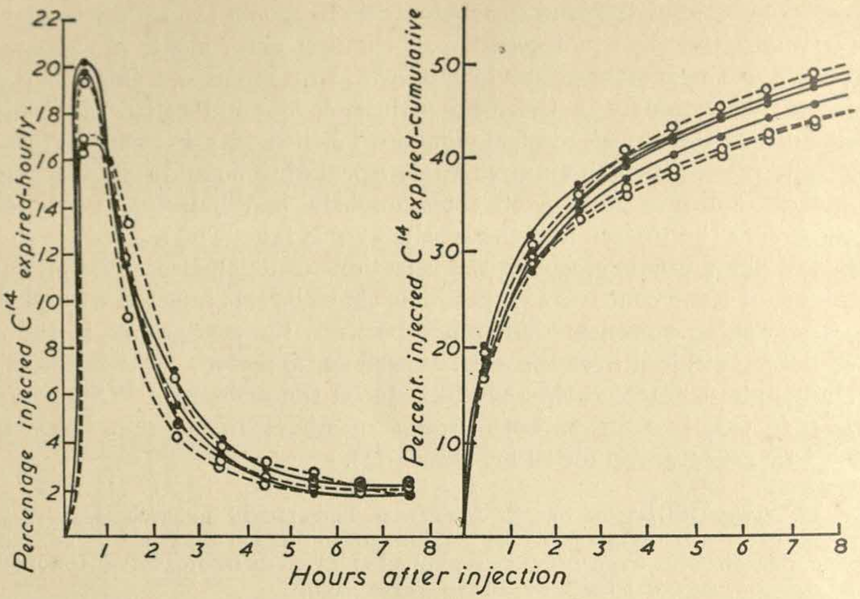

FIG. 5.—The effect of feeding carbohydrate or fat on the oxidation of ^{14}C labelled tripalmitin. The percentage of the injected ^{14}C tripalmitin oxidized to CO_2 is shown hourly and cumulatively over an 8 hr. period following the injection of 50 mg. of chyle fat into rats. Results for individual animals in each group are given.

○———○: fed glucose (1125 mg.).

●———●: fed olive oil (500 mg.).

40 per cent of the injected ^{14}C tripalmitin was oxidized in 8 hr. (Table II) and this was about 20 per cent less than the amount of tripalmitin oxidized by rats starved 24 hr.

DISCUSSION

The results presented indicate the rate at which the long chain fatty acids of tripalmitin are oxidized by the rat under physiological conditions. It is apparent that chylomicron fat is readily available for oxidation by the tissues. Only the rate of conversion of the fatty acid to the final oxidation product, CO_2, has been studied and no estimate can therefore be made of the other metabolic pathways which may be taken by the fatty acids. The extent to which the fatty acids were converted to CO_2 was dependent on the nutritional state of the animal and on the amount of fat presented for metabolism. The starved animals produced less CO_2 than the non-starved ones, which suggested either that these rats were already oxidizing more fat or had a lower metabolic rate. The increased significance of fatty acid metabolism in starvation is well recognized and when small amounts of fatty acid were injected into the starved rats it was oxidized to CO_2 very rapidly. When large amounts were injected, particularly in non-starved rats, a much greater proportion of the fat was passed into other metabolic channels presumably for storage as depot fat or conversion to other metabolic intermediaries.

The rates of oxidation obtained when tripalmitin has been injected in the form of an artificial emulsion are difficult to compare with the rates found in the present experiments in which homologous chyle was used. The rates vary with the amount of fat given, with the size of the animal and with the type of fat emulsion used. Waddell, Geyer, Saslaw and Stare [1953] showed that the different emulsifying agents used in the preparation of artificial emulsions affect the biological behaviour of the fat. Many of the emulsions used have contained glucose and this would alter the oxidation rate. The size of the emulsion particles may also be an important factor in determining the rate of oxidation of the fatty acids [Weinman, Chaikoff, Dauben, Gee and Entenman, 1950]. From the data available, however, it appears that, in the rat, tripalmitin, in the form of homologous chylomicron fat is oxidized more rapidly than when injected as an artificially stabilized emulsion.

The results obtained by Lossow and Chaikoff [1955] also indicate a difference between the metabolic behaviour of artificially emulsified fat particles and the chylomicra used in the present experiments. These workers showed that in the rat the oxidation of emulsified fatty acids was related to the availability of carbohydrate as a metabolic substrate. They found that carbohydrate exerted a sparing effect on the oxidation of fatty acids which was proportional to the chain length of the fatty acids. Feeding glucose reduced the oxidation of emulsified ^{14}C labelled tripalmitin and palmitic acid from 42 per cent to 5 per cent during a 6 hr. period following the injection of the labelled fat. It was concluded this was due to inhibition of the breakdown of the fatty acid molecule when glucose was available for oxidation. The experiments described here using homologous chyle labelled with ^{14}C tripalmitin confirm that the rate of oxidation of this fat is depressed in non-starved animals and by feeding glucose. However, glucose feeding reduced the percentage oxidized in 6 hr. only from 56 to 42 per cent. Moreover, the feeding of an isocaloric amount of fat reduced the oxidation of injected ^{14}C tripalmitin by about the same amount. When chylomicron fat or glucose was given intravenously there was similarly no difference in the extent to which the oxidation of ^{14}C tripalmitin was reduced. This suggested that both chylomicron fat and glucose were metabolized with approximately equal facility or possibly that a rapid conversion of chylomicron fatty acids to metabolic intermediaries which spared the oxidation of the labelled fat to CO_2 occurred.

The rates of oxidation of the labelled tripalmitin reported here were very close to those found for glucose by Zilversmit, Chaikoff, Feller and Masoro [1948]. These workers found that after the injection of 100 mg. of ^{14}C labelled glucose into non-starved rats about 28 per cent was converted to CO_2 in the 2 hr. following the injection. When an amount of ^{14}C tripalmitin of equivalent caloric value was injected into non-starved rats in the experiments described in this paper, about 26 per cent was oxidized to CO_2 in 2 hr. It appeared, then, that under the conditions described, both chyle fat and glucose were metabolized at about equal rates. When fat and glucose were given in isocaloric amounts either by mouth or by intravenous injection there was no evidence that the glucose was metabolized preferentially.

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USE OF SUBCUTANEOUS DEPOTS OF TEST SUBSTANCES IN THE MEASUREMENT OF RENAL CLEARANCES. By J. DUPRÉ and R. V. COXON. From the University Laboratory of Physiology, Oxford.

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An attempt has been made to evaluate the reliability of estimates of glomerular filtration rate and renal plasma flow based on determinations of the clearance of inulin and para-amino-hippurate (PAH) administered by subcutaneous injection. The results obtained in this way on four dogs were compared with the values established for the same animals by the use of continuous intravenous infusions of the test materials and good agreement was demonstrated. However, although the estimates of glomerular filtration rate showed satisfactory correspondence with published figures obtained by other workers, the present values for renal plasma flow appear somewhat high. In the case of man the use of subcutaneous injections on a group of four healthy subjects yielded figures for both inulin and para-amino-hippurate clearances which were well below the published results for larger series of determinations employing continuous infusions. Possible reasons for these divergences are discussed. Finally the effects of a pyrogen (typhoid-paratyphoid vaccine) and of adrenaline on renal clearances were examined in dogs and men respectively using subcutaneous depots of inulin and PAH. The responses seen resembled those reported in other studies where the continuous infusion technique was employed for making clearance measurements.

INTRODUCTION

THE somewhat elaborate arrangements required for giving continuous intravenous infusions at a constant rate as employed in the classical techniques [Smith, 1943] for carrying out determinations of glomerular filtration rate and renal plasma flow have stimulated many attempts at simplification. Such modifications, however, all suffer to a greater or lesser degree from the disadvantage that they sacrifice the chief merit of the continuous infusion, namely, the maintenance of a steady concentration in the plasma of the test material. Thus, in the case of a substance administered by a single intravanous injection the estimates of clearance have to be based on a steeply falling curve of plasma concentration, whose shape is not readily predictable since the clearance of the blood takes place not only through the kidney but also in a rather complicated fashion into the extravascular fluids of the body [Schachter, Freinkel and Schwartz, 1950]. Robson, Ferguson, Olrich and Stewart [1950 *a*] have endeavoured to circumvent this difficulty in the case of inulin by studying the kinetics of its penetration into the body-fluids after an isolated intravenous dose; the volume of distribution of this substance appeared to increase linearly with time over a period of two hours, and allowance for this permitted the calculation of a mean renal clearance over a given period once the rate of enlargement of the inulin space had been derived empirically for the particular subject. In practice a clearance period extending from ½ to 2 hr. from the injection was recommended by these authors, which makes the procedure unsuitable for studies of short-term changes in renal function.

Robson *et al.* [1950 *b*] have also evolved a similar method of arriving at the clearance and maximal excretory capacity for diodone but, as in the case of inulin, their calculated results were not checked against the infusion technique although they were consistent with published figures. Single intramuscular and subcutaneous injections, by introducing some delay in absorption of the test substance, would be expected to produce a less rapidly changing plasma level than single intravenous injections, and have in fact been used by a number of investigators in both the clinical [Bull, Jaekes and Lowe, 1950] and experimental [Pickford, 1947] field. There appears, however, so far as we can discover, to have been no systematic enquiry into the reliability of techniques which involve routes of injection other than the intravenous; we have therefore attempted such a check by comparing estimates of the inulin and para-amino-hippurate clearances obtained on the same small series of dogs both by subcutaneous injections of these materials and by continuous infusions. We have also investigated in dogs and men the feasibility of demonstrating by the subcutaneous method the effects on renal function of two pharmacological agents to which the response has been already defined by the continuous infusion procedure. Such agents are typhoid-para-typhoid vaccine and adrenaline [Smith, 1943].

METHODS

Chemical Analyses.—Inulin was estimated in a one in ten dilution of plasma, after precipitating the proteins with cadmium hydroxide, by the resorcinol method of Roe, Epstein and Goldsyein [1949]. Urine samples were suitably diluted and similarly analyzed. It was found that concentrations down to 0·5 mg./100 ml. (equivalent to a plasma concentration of 5 mg./100 ml.) could be accurately measured in this way and recoveries from twelve samples of plasma and urine from men and dogs ranged from 97 to 105 per cent. The inulin used was supplied by Messrs. Kerfoot of Bardsley, Lancs.

Para-amino-hippurate (PAH) in plasma and appropriately diluted urine was estimated by a modification of the method of Bratton and Marshall [1939] using the same protein precipitant as for inulin. The procedure was as follows: To 5 ml. of protein-free fluid was added 1 ml. of 1·2 N HCl followed by 0·5 ml. of 0·1 per cent sodium nitrite in water; after an interval of 3 to 5 min. 0·5 ml. of 0·5 g. per cent ammonium sulphamate was also added and after a further 3 to 5 min., 0·5 ml. of 0·1 g. per cent N-(1 naphthyl)-ethylenediamine hydrochloride. The colour developed could be measured with accuracy in solutions of concentration above 0·03 mg./100 ml. and was directly proportional to the concentration up to 0·15 mg./100 ml. This covered a working range of plasma concentration of 0·3–1·5 mg./100 ml., that is, well below that at which tubular secretory power becomes limiting. Recoveries from eight samples of plasma and urine from men and dogs ranged from 97 to 105 per cent with a mean of 100 per cent. The para-amino-hippurate was obtained from British Drug Houses Ltd.

Dogs.—The experiments were carried out on four mongrel bitches. The selected animal was fasted overnight before a test, and diuresis was induced by giving water (50 ml./kg.) by stomach tube. In prolonged experiments this dose was repeated as required to maintain diuresis. Urine, collected during accurately timed periods of 10 to 15 min., was obtained through an indwelling urethral catheter, and at the end of each collection period the bladder was washed out with at least 10 ml. of sterile saline. The passing of the catheter was facilitated by previous episiotomy. Urine and washings from each period were pooled for analysis. Blood samples were obtained at the middle of each clearance period by venepuncture.

For subcutaneous injections of inulin and sodium para-amino-hippurate the shoulder-area was used and the volumes introduced corresponded to about 1·5 ml./kg. body-weight. Given as a mixed neutral solution in water containing 20 g. inulin/100 ml. and 3 g. sodium para-amino-hippurate/100 ml., the injection fluid was approximately isotonic, and the introduction of about 10 ml. into a 7 kg. dog gave plasma concentrations around 7 mg./100 ml. for inulin and 1 mg./100 ml. for PAH, which were maintained for a period of over an hour. In the rather prolonged experiments necessary to establish the effect of typhoid vaccine, a second injection of inulin and PAH containing one-half of the original dose was given about 90 min. after it.

For continuous intravenous infusion, a mixed neutral solution of inulin and PAH in normal saline was prepared and was delivered from a mechanically driven syringe to a cannula inserted into a hind-limb vein. After a priming injection of 30 mg./kg. of inulin and 3 mg./kg. of PAH, the rate of infusion of the test substances was adjusted in each animal to balance their rates of excretion as indicated by the clearance values obtained by the subcutaneous injection method. In this way plasma concentrations similar to those produced by the subcutaneous injections were maintained.

The dogs remained conscious throughout both procedures and lay loosely restrained on an animal table.

Men.—In the experiments on man, inulin and PAH were administered by subcutaneous injection to subjects who had fasted overnight. It was hoped to give doses similar to those administered to the dogs, but the solution of 20 g./100 ml. inulin prepared in the laboratory caused considerable local irritation at the site of injection. A ten per cent solution made up in ampoules for injection by Messrs. Kerfoot was therefore used and the strength of this limited the dosage administered. The injections were made in the axillæ, and it was found that a volume of about 25 ml. was the greatest that could be carried with comfort in a single depot. The PAH for these experiments was obtained as a twenty per cent solution of the sodium salt, some from Messrs. May & Baker and some from Messrs. Sharp & Dohme, and the calculated quantity of this was added to the warmed inulin solution immediately prior to injection. The mixture so prepared was very nearly isotonic with tissue fluid.

Urine samples were voided voluntarily without catheterization. The subjects maintained a water-intake of 10 ml./min. during the experiments.

RESULTS

Table I illustrates the degree of constancy in plasma-level of the test-substances attained during the second hour following a subcutaneous injection in the human and canine subjects. The usual dose administered to the dogs was in the region of 0·3 g./kg. body-weight in the case of inulin and 0·025 g./kg. in the case of PAH, while the human subjects received 0·1 g./kg. and 0·025 g./kg. of inulin and PAH respectively. Fig. 1 gives a rather fuller picture of the time-course of the plasma-levels in a representative dog (fig. 1*a*) and man (fig. 1*b*).

TABLE I.—PLASMA CONCENTRATION OF INULIN AND PAH FOLLOWING SUBCUTANEOUS INJECTIONS

Subject	Experiment	Inulin (mg./100 ml.) Minutes after injection		PAH (mg./100 ml.) Minutes after injection	
		80	120	80	120
Dogs					
Touch	1	8·0	9·5	1·25	0·85
	2	8·8	10·4	1·59	1·09
Go	1	10·9	8·9	1·00	0·61
	2	9·7	..	1·17	..
	3	8·8	10·6	1·19	0·74
Susie	1	8·0	9·2	1·06	0·89
	2	7·8	8·5	1·16	0·71
	3	4·6	9·9	1·40	1·35
Bambi	1	5·8	5·9	1·17	0·80
	2	16·6*	16·9*	1·03	0·76
Humans					
A	..	16·1*	18·9*	2·27	1·70
B	..	5·9	6·6	1·09	0·70
C	..	2·7	2·8	1·07	1·08
D	..	4·2	5·4	1·57	1·14

* Double usual dose given.

In Table II are summarized the clearance values obtained for the dogs by both the subcutaneous injection and the intravenous infusion techniques. Table III gives the clearance values obtained in four men by the subcutaneous injection method. In calculating the clearances shown in Table II the practice of Smith, Goldring and Chassis [1938] was adopted for the purpose of arriving at a correction for "delay-time"; their practice (which will be referred to again in a later section of this paper) involves inserting into the

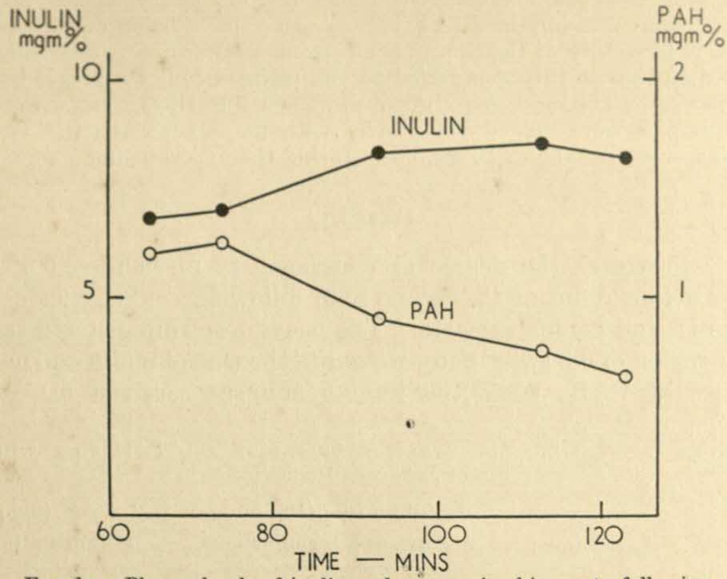

Fig. 1*a*.—Plasma levels of inulin and para-amino-hippurate following a subcutaneous injection of these materials in the dog.

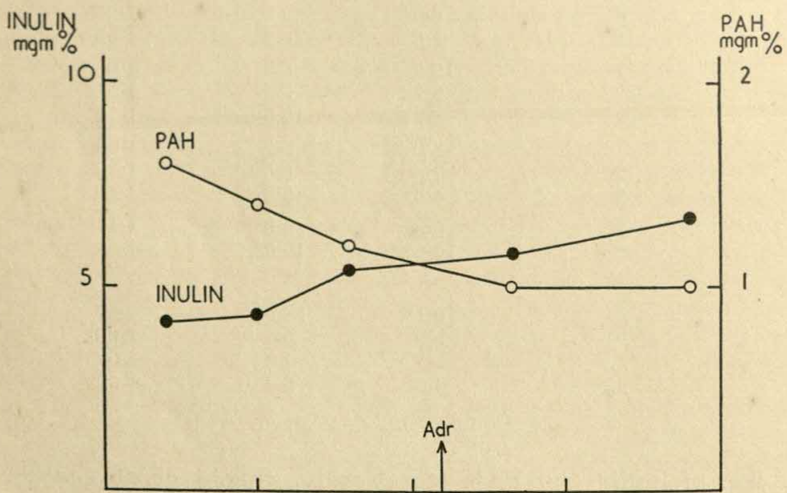

Fig. 1*b*.—Plasma levels of inulin and para-amino-hippurate following subcutaneous administration in man.

clearance equation a value for plasma concentration appropriate to a time preceding the mid-point of the urinary collection period by 150 sec. in the case of man. For the dog we used a correction of 100 sec. following Morales, Crowder, Maxwell, Fishman and Gomez [1950]. In our experiments the required plasma-concentrations were estimated by interpolation. Where a sufficiently constant plasma level of inulin or PAH was attained in an infusion experiment a separate estimate of clearance derived from the rate of infusion

TABLE II.—NORMAL VALUES IN DOGS

Subject	Experiment		Inulin clearance ml./min.	PAH clearance ml./min.
	No.	Type		
Touch, 8 kg.	1	Injection	26, 28	136, 138
	2	Injection	30, 28	131, 126, 138, 130
		Mean of above	28	133
		Mean/kg.	3·5	16·6
	3	Infusion	29, 31	115, 132
		From infusion rate	31	122
Go, 7 kg.	1	Injection	43, 33, 35	143, 133, 110
	2	Injection	41, 39	120, 108
	3	Injection	47, 50	119
	4	Injection	45, 51	149, 128
		Mean of above	42·7	126
		Mean/kg.	6·1	18·0
	5	Infusion	38, 39	156, 139
		From infusion rate	. .	131
Susie, 7 kg.	1	Injection	37, 35, 38	113, 112, 108
	2	Injection	38, 39, 32	110, 107, 106
	3	Injection	37, 33	92, 91
		Mean of above	36	105
		Mean/kg.	5·1	15·0
	4	Infusion	34, 35	108, 108
		From infusion rate	. .	102
Bambi 11 kg.	1	Injection	61, 58, 51	177, 189, 162
	2	Injection	47, 47	184, 176
		Mean of above	53	178
		Mean/kg.	4·0	16·1
	3	Infusion	45, 40	181, 188
		From infusion rate	. .	180
		Mean and S.D. per kg., for series (injection only)	5·19 ± 1·08	16·5 ± 1·80

TABLE III.—NORMAL VALUES, MAN
(ml./min./1·73 m.^{2})

Subject	Inulin clearance in successive periods	PAH clearance in successive periods
A	88, 77, 90	466, 428, 427
	Mean and S.D. = 85 ± 7·0	Mean and S.D. = 437 ± 23
B	110, 97, 108, 105	509, 445, 635, 471
	Mean and S.D. = 105 ± 5·7	Mean and S.D. = 515 ± 82
C	88, 101, 93	497, 511, 426
	Mean and S.D. = 94 ± 6·6	Mean and S.D. = 478 ± 40
D	105, 101, 106	300, 323, 304, 303
	Mean and S.D. = 104 ± 2·6	Mean and S.D. = 310 ± 11
All	Mean and S.D. = 96·5 ± 9·5	Mean and S.D. = 436 ± 89

was made as a check on the results computed from the urinary analyses and, where available, these figures have been included in the table.

The term "basal" in relation to Tables II and III implies that the determinations were made in resting subjects in the postabsorptive state. For comparison with other published figures the mean values are expressed per kg. body-weight for the dogs and per 1·73 sq. m. of body-surface for the men.

TABLE IV.—EFFECT OF TYPHOID-PARATYPHOID VACCINE IN DOGS

Subject: "Touch"	Time	− 21	− 11	+ 82	+105	+123	+155
Method: Injection	C In.	..	..	27	23	27	26
Dose 1·2	C PAH	137	131	163	166	159	122
Subject: "Touch"	Time	− 30	− 20	+ 50	+ 82	+116	..
Method: Infusion	C In.	29	31	30	34	31	..
Dose 2·4	C PAH	115	132	165	144	125	..
Subject: "Go"	Time	− 25	− 15	+115	+148	+169	..
Method: Injection	C In.	47	50	54	50	46	..
Dose 2·8	C PAH	119	..	151	136	122	..
Subject: "Go"	Time	− 33	− 10	+ 7	+ 69	+109	..
Method Infusion	C In.	38	39	38	48	51	..
Dose 2·8	C PAH	156	139	158	189	224	..
Subject: "Susie"	Time	− 25	− 9	+128	+152	+192	..
Method: Injection	C In.	37	33	32	27	26	..
Dose 2·8	C PAH	92	91	119	116	90	..
Subject: "Susie"	Time	− 22	− 11	+ 10	+ 60	+110	..
Method: Infusion	C In.	34	35	36	..	..	
Dose 2·8	C PAH	108	108	99	125	177	..
Subject: "Bambi"	Time	− 22	− 9	+115	+141	+166	..
Method: Injection	C In.	47	47	56	53	53	..
Dose 3·6	C PAH	184	176	219	211	187	..
Subject: "Bambi"	Time	− 15	− 5	+ 44	+ 75	+106	+131
Method: Infusion	C In.	45	45	45	47	53	47
Dose 3·6	C PAH	181	188	194	230	251	247

Time in minutes before (−) or after (+) administration of vaccine.
Dose of vaccines in organisms/kg. dog.
C In. and C PAH in ml./min.

Table IV shows the renal responses to typhoid-paratyphoid vaccine (TAB) in the dogs as demonstrated by both the injection and infusion methods. The vaccine was administered intravenously and, in accordance with established custom, antipyrin (25 mg./kg.) was given by mouth about 2 hr. prior to the injection of vaccine in order to minimize the degree of fever produced.

Table V gives the findings in two experiments in which the renal effects of adrenaline were studied in two healthy men by means of the subcutaneous injection method for determining clearances. The adrenaline was given in two equal doses of about 0·015 mg./kg., the first being administered subcutaneously and the second intramuscularly. In Table V the maximal changes in the clearances which were observed are indicated as percentages of the corresponding mean basal values.

TABLE V.—EFFECT OF ADRENALINE ON HUMAN SUBJECTS (INJECTION METHOD)

Subject (dose/kg.)	Time in minutes (−before +after adrenaline)	Inulin clearance ml./min.	PAH clearance ml./min.	Filtration fraction
C	−47	106	601	0·17
(0·013 mg.)	−33	122	619	0·20
	−19	112	515	0·22
	+ 6	110	432	0·25
	+32	..	422 (−27%)	..
D	−51	..	350	..
(0·015 mg.)	−36	123	376	0·33
	−23	118	355	0·33
	−10	125	354	0·35
	+11	109	289 (−19%)	0·38
	+33	112	359	0·31

DISCUSSION

Our most direct comparison of the absolute values given by the two methods tested is founded upon the basal figures obtained in our dogs. Since the data for the injection method are more numerous, the differences between clearances determined by the two methods can be conveniently expressed as percentages of the average value found by the injection method. In the case of inulin the mean and standard error of these differences were 13 ±7 per cent and in the case of PAH were 4 ±7 per cent. Thus, neither of these differences is significantly different from zero, and further, since they are actually of opposite sign, they are most unlikely to reflect any systematic divergence. A second but less direct comparison which is of interest is that between the values which we obtained by the injection method and those recorded for a series of 75 infused bitches by Houck [1948]. As indicated in Table III, the mean and S.D. of our injection results calculated on a body-weight basis were 5·19 ±1·08 ml./min./kg. for the inulin clearance and 16·5 ±1·80 for the PAH clearance; in Houck's series corresponding figures were 4·29 ±1·01 ml./min./kg. for creatinine (which should be equal to the inulin figure in a dog) and 13·51 ±3·26 for PAH. There is no significant difference between Houck's and our estimates of glomerular filtration rate (GFR) but the difference between the estimates of effective renal plasma flow (RPF) is highly significant as judged by "Student's" 't' test [Fisher, 1950]. However, in the case of PAH clearance our figures are well within the extremes of the range quoted by Houck and, although the difference revealed by the "t" test cannot be ascribed solely to unequal variances in the two series, the discrepancy is in the wrong direction to be explained by any of the physiological considerations to be discussed in connexion with our human results, and it therefore remains somewhat of a mystery. The coefficient of variation within our series is very close to that in the larger series so far as GFR is concerned and apparently smaller in the case of PAH clearance, but the precision of such a comparison is limited by the figures for the body-weights of the dogs which, in our series, were taken only to the nearest kilogram.

When the human figures obtained by the subcutaneous injection method and recorded in Table III are compared with results published by other workers who have used the constant infusion technique, considerable disagreement is at once apparent. Thus, when our figures for either GFR or effective RPF are set alongside those quoted by Homer Smith [1951] or by Shock and Davies [1950] they are found to be lower by a margin which is likely to arise by chance only once in more than 1000 trials. (Although our subject "D" is known from other evidence to show a low PAH clearance, even when his figures are omitted from the calculation of the mean, the differences from other published results remain.) Thus, the mean and standard deviations of the values for our four subjects obtained by the subcutaneous depot technique were 96·5 ±9·5 ml./min. for inulin clearance and 436 ±89 ml./min. for PAH clearance while the corresponding figures quoted by Homer Smith [1951] for a series of subjects studied by continuous infusion were 124 ±25·8 and 654 ±163 respectively.

In seeking to account for these differences two possible contributory factors come readily to mind. The first of these is the effect of a changing plasma concentration of the test-substances for it has been clearly demonstrated by Brun, Hilder and Raaschou [1949] that the clearance of diotrast, if determined while the plasma-concentration is declining, may fall short of that measured on a rising curve by as much as fifty per cent. These authors conclude that, apart from the obvious uncertainties involved in selecting under these circumstances a proper plasma value for insertion in the equation used in calculating clearance, the disparity between the arterial and venous concentrations introduces an appreciable error, since it is the former which determines the renal load while it is the latter which is usually measured. The same considerations apply, in lesser degree, to inulin. Furthermore, Newman, Ketton, Genecia, Genest, Calkins and Murphy [1949] have reported that the clearance of PAH tended to remain persistently low for a lengthy period following an abrupt fall in plasma concentration, even if this concentration had become stabilized by the time the actual measurements were made. They interpreted this finding, which was applicable to human but not to canine subjects, as indicative of an altered capacity on the part of the renal tubules to acetylate PAH, but their argument is weakened by the fact that the existence in their experiments of a similar relationship in the case of diatrast compelled them to postulate some comparable chemical transformation affecting this substance also. However this may be, the rates of change of plasma PAH in our own subjects were much less rapid than in the cases in whom Newman *et al.* found a depression of clearance, and, in fact, approximated to their criteria for "constancy". It is therefore very doubtful if the fluctuations in blood-concentrations of inulin and PAH can provide a sufficient explanation of the low clearances which we observed.

A second factor which may affect our results is the rather marked fall in the rate of urine formation which occurred during the course of the experiment in two of our subjects, and which, according to Homer Smith [1951], may be associated with a sharp decrease in all clearance values. This antidiuretic effect which followed the subcutaneous injections was attributed by us to the transient discomfort and more prolonged apprehension occasioned by them. In this connexion a second observation of Homer Smith [1951] may also be relevant, namely that there is a tendency for all clearances to drift downwards in lengthy experiments, this drift being, as he says, loosely attributed to discomfort and fatigue among other possible causes. However, among our four subjects the two who showed no antidiuretic response gave clearances which were not consistently higher than those of the other two subjects so that this complicating circumstance does not appear to have much importance in explaining our results. The lowness of our figures as compared with those obtained in larger series of studies by the theoretically sounder procedure of continuous infusion cannot therefore be very convincingly explained away, but it is notable that in the long list of values cited by Homer Smith [1951] those derived from subcutaneous injections of diotrast appear consistently lower than those determined by continuous infusion in the case of male subjects. This, however, is not so in the case of female subjects, while the inulin values by the two methods do not differ in either sex. It is also notable that in the small number of values for normal subjects reported by Robson *et al.* [1950 *a* and *b*] which were measured after single intravenous injections, rather low figures for inulin and diotrast clearances were obtained.

A further source of uncertainty which assumes some importance when computing clearances in the presence of fluctuating plasma-levels of the test substance is connected with the so-called "delay-time". This is the interval elapsing between the elaboration of a particular quantum of urine in the nephron and the arrival of its contained solutes at the point of sampling. The problem of estimating this interval has attracted the attention of a number of workers in recent years and there is some question as to the validity of the conventional corrections which we employed. Thus, the work of Chinnard [1955] suggests that the appropriate "delay-time" correction in dogs weighing 6 to 13 kg. might be nearer to 3 min. than to 1·5, while the work of Childs, Wheeler, Ciminsky, Leifer, Wade and Bradley [1955] similarly suggests that 6 rather than 2·5 min. may be an acceptable figure for the average man. However, recalculation of our results using these modified corrections does not materially affect our final figures.

Analysis of the variance among our results shows that, so far as the dogs are concerned, the variance between individuals is very much greater than that between different clearance periods in the same animal ($P < 0·01$). As regards human subjects this also applies but the exclusion of subject "D" from the analysis of the PAH results removes the difference in that instance. The variance between periods in the same human subject is for inulin almost identical with that noted by Shock and Davies [1950] in thirteen subjects of roughly the same age, but our PAH results show a wider scatter than theirs, the S.D. being ±54 ml./min. as compared with ±33 ml./min. Our subject "D", who was the oldest of the four, conformed to the age-pattern described by Shock and Davies in having both a lower and a more constant PAH clearance.

Turning to the experiments on the response to pharmacological agents the picture is considerably clearer and more encouraging in regard to the subcutaneous technique. Thus the results of studying the effect of typhoid-paratyphoid vaccine set out in Table IV show that the expected rise in renal plasma flow was clearly demonstrated in all four dogs by both injection and infusion procedures. The magnitude of the changes seen cannot, however, be readily compared in any two runs owing to different time-courses, apart from other sources of variation. In the two men in whom the effect of adrenaline was studied the response elicited was that predicted from Homer Smith's [1943] earlier studies using the continuous infusion technique. Unfortunately, in the case of subject "C" the PAH clearance was already falling before the adrenaline was given but it fell more strikingly afterwards. The subjects receiving adrenaline, although well practised in the experimental procedure, had some difficulty in emptying their bladders completely—due presumably to over-active sphincters—but they appeared to overcome this successfully as is confirmed by the relative constancy of their outputs of inulin. Reliance upon voluntary micturition is, of course, a departure from the ideal technique for collecting urinary samples, but has been adopted by others [Pitts, Ayer and Schiess, 1949].

Other possible circumstances, which might contribute to the divergences between our human results and the values in the literature, are the influence of the subject's posture and of the introduction directly into his circulation of isotonic fluid, unavoidable with continuous infusion. From the nature of the apparatus to which he is connected the subject of a clearance determination by the infusion method is generally relatively immobile and is often recumbent. Subjects studied by a single injection may on the other hand remain active and ambulant. Assumption of the recumbent posture does, in fact, appear to reduce both the glomerular filtration rate and the renal plasma flow in some individuals but of a series of twelve persons studied by McCance [1951] only four showed these changes. The effect of posture is therefore unlikely to provide a general explanation of the low figures obtained by single injection techniques. The question of the effect of the infusion is less easy to settle although the administration of the same quantities of inulin and PAH in different volumes of saline could clearly be tested in this connexion. However, it seems that infusions of saline sufficiently large to exert a marked influence on the rate of urine flow did not, in the experiments of Strauss, Davies, Rosenbaum and Rossmeisl [1951], affect endogenous creatinine clearance. Since the rate of infusion of saline in their subjects was about six times greater than that employed in the determination of renal clearances by Smith *et al.* [1938], here again the likelihood of the infusion itself being a complicating factor is very slight.

Our human studies would have been rendered easier from the standpoint of the chemical analyses had there been available a non-irritant twenty per cent solution of inulin which would have permitted a rather larger dose of this material, but the general trend of the results would probably not have been altered.

We feel justified in concluding from the present experiments that the use of subcutaneous injections for clearance studies on dogs may be expected to give reasonably satisfactory results for inulin and PAH in terms both of the numerical values obtained and the changes which can be demonstrated. In the case of man, the numerical results are significantly different from published estimates based on the continuous infusion method but, judged by the response to adrenaline in our subjects, the clearances measured after subcutaneous injections do successfully indicate changes in renal behaviour. The use of subcutaneous injections in both dogs and men avoids the necessity for a constant-speed infusing apparatus; it also conserves veins when the same subjects are repeatedly studied and permits greater mobility of the subject during the course of a run.

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THE INFLUENCE OF POSTURE ON THE DIURETIC RESPONSE TO THE DRINKING OF ISOTONIC SALINE.* By R. V. Coxon, J. Dupré and R. J. Robinson. From the University Laboratory of Physiology, Oxford.

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The rate of urine flow following ingestion of isotonic fluid has been compared in ambulant and recumbent human subjects. The majority of recumbent subjects showed a more marked diuresis than did their opposite numbers in the ambulant group. Possible explanations of this postural effect are discussed.

Introduction

The simple experiment reported here was undertaken in order to throw light on some apparent inconsistencies in the published accounts of the effects on urine flow of the administration of isotonic salt solutions. Douglas and Priestley [1948] state that the diuresis which follows the drinking of a moderate volume (*c*. 500 ml.) of water is not seen following the ingestion of a similar volume of isotonic salt solution and this has been our usual experience during a large number of class experiments. On the other hand, Blomhert, Gerbrandy, Molhuysen, de Vries and Borst [1951] report that the drinking of some 2 litres of "extracellular fluid" is attended by a diuresis which is similar to that induced by taking a comparable volume of water. Apart from the difference in volumes of fluid taken it seemed that the posture and state of activity of the subjects might enter into the overall response and we therefore arranged to compare the results of giving salt solution in equal quantity to two similar groups of people, one group remaining ambulant while the other group rested on couches during the period of urine collection. The clear-cut differences between the two groups in terms of their urine output form the substance of the present communication.

Methods

Each subject drank a dose, representing 10 ml./kg. of his body weight, of the fluid whose composition is given in Table I alongside published data for the composition of extracellular fluid. After taking the dose of fluid, one group of subjects immediately lay down on couches and remained there for the full 2 hr. during which urine samples were collected without rising more than was absolutely unavoidable from the supine position. The other group of subjects carried out the normal activities of a practical class in electrophysiology except for retiring as necessary in order to produce urine samples. The urine specimens voided at 20-min. intervals were measured for volume, and the specific gravities were also determined either by a gradient tube or standard urinometer, depending on the amount of material available. The subjects were all healthy male undergraduates.

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* A brief account of this work was communicated to the Twentieth International Physiological Congress in Brussels, 1956.

The osmolarity of the salt solution drunk was compared with that of normal serum by a cryoscopic method, and the results are included at the bottom of Table I.

TABLE I.—COMPARISON OF SOLUTES

(m.equiv./l.)

Ion	Beverage	E.C.F.
Na^+	152	145
K^+	5·8	3·3
Mg^{++}	2·8	2·4
Ca^{++}	5·6	3·2
Cl'	124	111
HCO_3'	40	28
HPO_4''	2·2	2·0

Osmolarity of beverage = 0·285. S.G. = 1005 at 20° C.
Osmolarity of human serum = 0·300.

E.C.F. = extracellular fluid based on data of Darrow [1945] and Danowski [1955].

The "P" values in Table II were calculated by applying "Students t" test to the difference between means [Fisher, 1950]. There were twelve subjects in the ambulant group and eight in the recumbent group.

RESULTS

The mean rates of urine flow in the two groups of subjects at intervals after the loading dose of fluid are shown in Table II. At the peak of the diuresis the mean rate of urine flow in the recumbent subjects was about ten times their resting output while in the ambulant group the corresponding rate was only three times the resting figure. The differences between the mean flow-rates in the two groups during this and the three subsequent periods were statistically significant.

TABLE II.—COMPARISON OF URINE FLOW IN RECUMBENT AND AMBULANT SUBJECTS

Time from drinking load (minutes)	Mean urine flow (ml./min.) ±S.D.		Difference	S.E. of difference	P.
	Recumbent (n = 8)	Ambulant (n = 12)			
Before	0·8 ± 0·4	0·8 ± 0·4	0	..	..
0–20	1·3 ± 0·7	1·7 ± 1·5	0·4	0·95	> 0·7
20–40	4·6 ± 5·2	2·8 ± 2·6	1·8	2·13	> 0·3
40–60	7·9 ± 5·6	2·4 ± 3·6	5·5	2·38	< 0·05
60–80	5·4 ± 3·1	1·3 ± 0·4	4·1	1·17	< 0·01
80–100	6·5 ± 5·6	1·4 ± 0·6	5·1	2·12	< 0·05
100–120	2·9 ± 1·7	1·4 ± 0·3	1·5	0·66	< 0·05

n = number of subjects.

Examination of the flow-rate for individual subjects shows that three of the ambulant subjects had a moderate diuresis during the first hour of the experiment but in all cases this had passed off before the second hour's collections began. One of the recumbent subjects showed no diuresis during the 2 hr. when he was under observation. The course of events in these atypical subjects is shown in Table III.

TABLE III.—RESPONSE OF ATYPICAL SUBJECTS

Time from drink (minutes)	Urine flow in ml./min. Subject A ambulant	Subject D ambulant	Subject E ambulant	Subject N recumbent
Resting	1·4	0·7	0·9	0·5
0–20	1·4	0·9	6·2	0·8
20–40	1·4	5·8	9·8	1·7
40–60	13·6	2·2	0·6	1·9
60–80	1·7	1·4	0·6	1·4
80–100	1·7	2·1	2·9	1·8
100–120	1·7	1·0	1·6	1·6

Table IV illustrates the changes in urinary specific gravity which occurred along with the increase of urinary flow in a typical recumbent subject.

TABLE IV.—RELATION BETWEEN SPECIFIC GRAVITY OF URINE AND RATE OF FLOW IN A RECUMBENT SUBJECT

Time from drinking	Flow (ml./min.)	Specific gravity
Rest	0·4	1030
0–20	0·7	1032
20–40	2·2	1022
40–60	8·5	1000
60–80	9·0	1002
80–100	14·0	1004
100–120	5·5	1010

DISCUSSION

The findings reported above are complementary to those of Strauss, Davies, Rosenbaum and Rossmeisl [1951] who found that the intravenous infusion of isotonic saline solutions provoked a diuresis in recumbent but not in sitting subjects and to those of McCance [1951] who demonstrated an increased diuretic response to water in the lying as against the standing subject. These postural effects are all related to the question of the possible existence of receptors sensitive to the volume as distinct from the tonicity of the body fluids and capable of adjusting the rate of urine flow—presumably through the hypothalamus and posterior hypophysis. Peters [1935] in a much quoted passage mentioned receptors signalling "fulness of the blood-stream" but more recently many attempts [see Drury, 1955] have been made to reveal the localization and neural connexions of such receptors. Among the more direct of these are the experiments of Henry and Pearce [1956] who showed (admittedly in anæsthetized and considerably traumatized dogs) the existence of vagal afferent fibres which appeared to respond to stretching of the walls of the right atrium and whose activation seemed to be followed by an increased rate of urine flow. These authors concluded that such fibres might be excited by any circulatory change which would result in the accumulation of blood in the thoracic venous channels. Both the assumption of a reclining posture and also breathing against a subatmospheric pressure, would tend to favour the accumulation of blood in that situation and might exert their known effects on urine flow through such a mechanism. In the case of a subject absorbing isotonic salt solution from the alimentary tract an expansion of the blood volume accompanied by a somewhat later increase in the extracellular fluid volume may be expected. A mechanism reacting to distension of the right side of the heart and adjacent veins could therefore be activated by the blood-volume change provided this was sufficiently great and sufficiently maintained. In this connexion the early observations of Haldane and Priestley [1916] appear relevant. They showed that hæmodilution of the order of 5–10 per cent was detectable about 1 hr. after the ingestion of the same salt solution as was used by us. Since Henry and Pearce state that the atrial receptors studied by them were sensitive to changes of about 5 per cent in the dog's blood volume it seems likely that these receptors could be stimulated by the ingestion of saline. It should be pointed out, however, that Haldane and Priestley's measurements of hæmodilution were made following the ingestion of considerably larger quantities of salt solution than were taken by our subjects. On the other hand, it seems likely that the rate of absorption may not have been vastly different in the two cases since Haldane and Priestley [1916] recorded that in an experiment in which they sought to promote a maximal turnover of fluid the limiting rate of absorption and excretion appeared to be in the region of 700 ml./hr. This was about the average dose taken by our subjects and if it were mostly absorbed in the first hour and mostly excreted in the second hour, the blood-volume change at the end of the first hour could be of the order of 7 per cent in the average subject. In fact, among our recumbent subjects the highest urine flows were generally found between the fortieth and hundredth minutes of the run. Strauss *et al.* [1951] in their study of the diuretic effect of intravenous saline used an infusion rate of 1500 ml./hr. and, although their data on urine flow are tabulated only by hourly or longer intervals, it seems that the diuretic effect was most clearly marked after an hour or more had elapsed from the start of the infusion. The expansion of blood volume by this time would probably be greater than in our subjects, but, since such an expansion would be determined ultimately by the relative rates of entry into and removal of fluid from the vascular bed the balance between the two may be differently affected according to the route by which the process of entry takes place.

McCance [1951] in summarizing the evidence regarding the diuresis which is associated with recumbency in undernourished persons and gives rise to the nocturia that they frequently experience, comments that this may be a result of the expansion of the extracellular fluid volume which is known to occur in such persons.

As happened in men studied by Priestley [1916], our subjects also at times (*cf.* Table IV) produced urine which was of somewhat lower specific gravity than the ingested fluid, thus indicating a temporary dissociation of the homæostatic adjustments to the solvent and solutes introduced into the body.

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INDUCTION OF TOLERANCE TO HOMOGRAFTS OF THYROID AND ADRENAL IN RATS. By M. F. A. WOODRUFF* and MARGARET SPARROW. From the Department of Surgery, University of Otago, Dunedin, New Zealand.

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A technique is described for measuring the uptake and release of ^{131}I from thyroid grafts in rats using a collimated scintillation counter. These measurements provide a useful means of assessing the amount of surviving thyroid tissue and its functional activity. Homografts of thyroid, placed alongside the femoral vessels in thyroidectomized rats which were injected at birth with spleen cells from the same donor, are as a rule indistinguishable from autografts both in their capacity to take up ^{131}I and in their histological structure, whereas homografts in thyroidectomized but otherwise untreated hosts are usually destroyed within 6 weeks. Homografts of whole adrenal glands sutured to the ovaries of adrenalectomized recipients which were injected at birth with donor spleen cells also behave like autografts. Homografts in adrenalectomized control hosts are sometimes destroyed in a few weeks, but sometimes survive for months, though they usually show evidence sooner or later of replacement of cortical tissue by connective tissue. The significance of these observations is discussed in the light of current views on transplantation immunity and immunological tolerance.

WOODRUFF AND SIMPSON [1954, 1955 *b*] reported that rats, injected subcutaneously on the day they are born with homologous spleen cells in suitable dosage, are, when they grow up, almost completely tolerant of skin homografts from the same donor. It seemed important to determine whether tolerance induced in this way extended to homografts of other tissues, and in the present investigation homografts of two endocrine tissues, thyroid and adrenal, have been used for this purpose.

METHODS

General plan of the Experiment.—Four litters of new-born Wistar rats were obtained from stock and animals in excess of eight in each litter were destroyed, care being taken to retain an even number of each sex. The animals were kept in individual cages in a thermostatically controlled room maintained at a temperature of 70° F. They received the standard laboratory diet of rat cubes, the composition of which was as follows:

Whole wheat meal	30 parts
Meat meal . .	24 parts
Pollard . .	24 parts
Milk powder .	18 parts
Bran . . .	12 parts
Maize meal . .	12 parts
Bone meal . .	2 parts
Cod liver oil .	1 part

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Except where the contrary is stated water was allowed *ad lib*. Each animal was given a distinguishing mark, initially by tattooing with Indian ink, and later, when the hair had grown sufficiently, by using dyes of various colours.

Each of the reduced litters was subdivided into four pairs of animals of like sex. One member of each pair received a subcutaneous injection of a suspension of spleen cells from an adult hooded rat of the same sex, a separate donor being used for each recipient. The other member of the pair was kept as a control and, in addition to bearing its own particular mark, was further distinguished by having the tip of its tail crushed.

When they were 12 weeks old, each of the injected animals received a homograft of either thyroid or adrenal from the original cell donor, and each of the control animals received a homograft of thyroid or adrenal from a stock hooded rat of the same sex. The choice of tissue was determined by the sex of the recipient, males receiving thyroid grafts and females adrenal grafts (Table I).

TABLE I.—GENERAL PLAN OF EXPERIMENT
(For explanation see text)

Litter No.	Pair No.	Sex	Laboratory numbers of members of pair		Type of cell suspension	Type of graft
			Injected	Not injected		
1	1	Male	427	428	Weak	Thyroid
	2	Male	430	431	Weak	Thyroid
	3	Female	433	434	Weak	Adrenal
	4	Female	436	437	Weak	Adrenal
2	5	Male	439	440	Weak	Thyroid
	6	Male	442	443	Weak	Thyroid
	7	Male	445	446	Weak	Thyroid
	8	Female	448	449	Weak	Adrenal
3	9	Male	451	452	Strong	Thyroid
	10	Male	454	455	Strong	Thyroid
	11	Male	457	458	Strong	Thyroid
	12	Female	460	461	Strong	Adrenal
4	13	Male	463	464	Strong	Thyroid
	14	Female	466	467	Strong	Adrenal
	15	Female	469	470	Strong	Adrenal
	16	Female	472	473	Strong	Adrenal

For comparison thyroid and adrenal autografts were studied in male and female Wistar rats respectively.

Preparation and Dosage of Cell Suspensions.—Cell suspensions were prepared by removing approximately one-third of the spleen of each adult (250–300 g.) hooded donor under ether anæsthesia, rubbing it gently in a glass homogeniser with either 1·5 ml. (giving a *weak* suspension) or 0·75 ml. (giving a *strong* suspension) of buffered Ringer's solution, and filtering through a standard blood transfusion filter of stainless steel mesh. Strong suspensions, like the suspensions used in the experiments on tolerance to skin homografts reported previously [Woodruff and Simpson, 1954, 1955 *b*; Woodruff, 1957], contained 150,000–200,000 nucleated cells per cu. mm.; weak suspensions 75,000–100,000 nucleated cells per cu. mm.

The dose used was 0·1 ml., *i.e.* 15–20 million cells or 7·5–10 million cells accordingly as a strong or weak suspension was used. The injections were given subcutaneously.

Thyroid Grafts

Autografts.—Total thyroidectomy was performed under ether anæsthesia. Animals in which the recurrent laryngeal nerves were accidentally injured were destroyed and replaced by others. The thyroid was cut up into sixteen pieces* and these were grafted to the right thigh in close proximity to the femoral vessels.

Homografts.—The donor's thyroid was removed and placed in a sterile Petri dish. A swab which had been dipped in boiling water was placed in the dish to keep the atmosphere moist, but was not allowed to come in contact with the tissue. The recipient's thyroid was then removed completely and discarded, after which the donor's thyroid was cut up into sixteen pieces and these were grafted to the right thigh of the recipient in close proximity to the femoral vessels. Both donor and recipient were anæsthetized with ether.

^{131}I Uptake Studies.—These were performed on all the recipients 6 weeks after grafting. If there was no evidence of function in the graft, the animal was killed and a search made for any remains of the graft; otherwise, further tests were performed 10 weeks and 16 weeks after grafting. At the conclusion of the last test the animal was killed and the graft removed for histological examination.

On the morning of the first day of the test each animal was given 0·2 ml. of a solution of iodide containing 5 μc. of carrier-free ^{131}I by intraperitoneal injection.† *In vivo* counts were made under light ether anæsthesia at the site of the graft, and also at the normal site of the thyroid gland in the neck, using a collimated scintillation counter.‡ As a rule the counts were made 24, 48, and 72 hr. after the injection of ^{131}I, but occasionally some of these counts were omitted, and in some of the animals which received autografts additional counts were performed 8 and 96 hr. after injection.

The dimensions of the collimator are shown in fig. 1. The position of the animal was adjusted by trial and error to give the maximal count (and for this purpose it was often convenient to scale down only 1:16, 1:8 or even 1:4). A definitive count was then made over a period of 1 min. (scaling down 1:64).

In order to be able to calculate from the *in vivo* counts what proportion of the injected iodine was taken up by each graft, a method of calibration was used similar in principle to that described by Brown-Grant, von Euler, Harris and Reichlin [1954]. Three animals bearing actively functioning autografts were killed immediately after the 72-hr. count of the last uptake test, and each graft was removed and digested by incubating it overnight at 37° C. in a standard source bottle containing commercial pepsin made up with distilled water to a volume of 0·4 ml. Counts were then made of each bottle with the scintillation counter and corrected to the time of the last *in vivo* count. The ratio, R, of the *in vivo* count to the corrected *in vitro* count was then calculated for each graft.§ Another source bottle of the same dimensions containing a known amount of ^{131}I in 0·4 ml. fluid was counted, and the *in vivo* count corresponding to 100 per cent absorption of the injected ^{131}I by the graft was then calculated as shown in Table II.

Tests for Completeness of the Thyroidectomy.—*In vivo* counts were made routinely over the normal site of the thyroid gland, and a search was made post-mortem for regenerating thyroid tissue. In addition the larynx with the tissue adjacent to it was removed *en bloc* from five animals in which there did not appear to be any regenerating thyroid on naked eye inspection, and tested with a β-sensitive Geiger-Müller counter.

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* These pieces are referred to collectively as a graft.

† Goldberg, Chaikoff, Lindsay and Feller [1950] found no evidence of damage to the thyroid gland or other tissues of the rat up to 18 months after a single injection of 18 μc. ^{131}I. It was thought therefore that 5 μc. would be a safe dose even for repeated testing on three or four occasions, and this proved to be the case.

‡ Counter made by the Nuclear Research Corporation. Reference number SC—1B. The collimator was very similar to that described by Brown-Grant and Gibson [1955].

§ The value of R depends on differences in the physical dimensions of the systems used for the *in vivo* and *in vitro* counts, and has no biological significance.

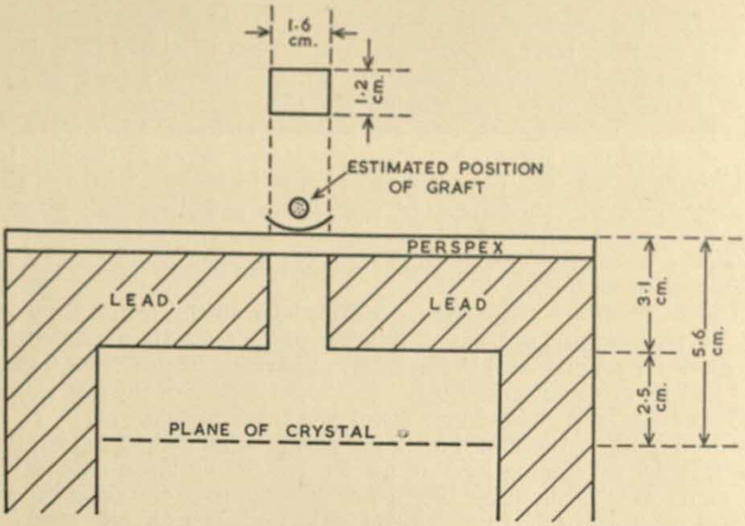

Fig. 1.—Dimensions of the collimated scintillation counter. The drawing is to scale only in respect of the dimensions indicated.

Table II.—*In Vivo—In Vitro* Counts of Thyroid Autografts following Intraperitoneal Injection of 5μc, ^{131}I

Animal No.	681	684	685
Interval between ^{131}I injection and *in vivo* count. Hours	72	72	72
In vivo counts per second	46.7	65.6	31.6
Interval between *in vivo* and *in vitro* counts. Hours	22.5	22·5	22·5
Observed *in vitro* counts per second	35·9	50·5	24·3
In vitro count corrected to time of *in vivo* count	38·9	54·6	26·3
$R = \frac{\textit{In vivo}\text{ count}}{\text{Corrected }\textit{in vitro}\text{ count}}$	1·20	1·20	1·20

Mean value of R = 1·20.

Counts per second obtained from 2·08 μc ^{131}I in standard bottle = 100·75.

Counts per second expected from 5 μc ^{131}I in standard bottle $= \frac{100\cdot75 \times 5}{2\cdot08} = 242\cdot2$.

In vivo counts per second corresponding to 5 μc ^{131}I in graft = 242·2 × 1·20 × 290·6.

In vivo counts per second 24 hr. after injection of ^{131}I corresponding to 100 per cent uptake of injected iodine = 290·6 × decay factor for 24 hr. = 290·6 × 0·912 = 265·0.

Adrenal Grafts

Autografts.—Under ether anæsthesia the left adrenal was removed through a dorsal incision and the whole gland was anchored to the left ovary by a single silk stitch. The right adrenal was then removed and attached to the right ovary in a similar manner. After the operation the animals received the standard diet, with 1 per cent sodium chloride *ad lib.* instead of water for 4 weeks.

In three animals the right ovary and graft was removed after 4 weeks, and the left ovary and graft after 16 weeks. In three other animals both ovaries and grafts were removed after 16 weeks. In every case the stitch anchoring the graft to the ovary was removed, after which the graft was dissected free, fixed in Bouin's solution, and sectioned. Each animal was killed when the second graft was removed.

Homografts.—The donor's adrenals were removed and placed in a sterile Petri dish containing a warm moist swab. The host's adrenals were then removed and discarded. One of the donor's adrenals was anchored to the left ovary of the host by a single silk stitch, the other to the right ovary in a similar manner. After operation the animals received the standard diet, but for 4 weeks thereafter they were given 1 per cent sodium chloride *ad lib.* instead of water.

The left ovary and graft were removed after 8 weeks, and the right ovary and graft after 16 weeks if the animal survived for this length of time. Provided the graft could still be recognized macroscopically, it was separated from the ovary in the same manner as an autograft (see above), fixed in Bouin's solution and sectioned.

After removal of the second graft the animal continued to receive the standard diet with water to drink until death.

RESULTS

Thyroid Grafts

General Observations.—The counts obtained over the grafts, corrected for laboratory background and expressed in counts per second, are set out in Tables III, IV and V. In twenty tests with autografts, counts were made 8 hr. after ^{131}I injection in addition to counts at 24, 48 and 72 hr. The ratios of the later counts to the 8-hr. count have been calculated for each test and the mean values have been plotted in fig. 2. In thirteen tests, the 24-hr. count was the highest; in the remainder the 8-hr. count was higher than the 24-hr. count. The ratio of the 24-hr. count to the 8-hr. count ranged from 0·70 to 1·67, with a mean of 1·03 and standard deviation ±0·20. These figures indicate that the 24-hr. count is a reasonably good approximation to the true maximum count, and it has been used generally for calculating the proportion of injected iodine taken up by the grafts in the light of the *in vivo—in vitro* tests.

The results of these calculations are shown in Table VI. In the first test, nine grafts, comprising eight homografts in control hosts and one homograft in a cell-injected host, yielded counts of less than 6·5 per second at 24 hr. These animals were killed and in none of them was any thyroid tissue found at the graft site. No 24-hr. counts in the range 6·5–13 per second were recorded. Counts of 13 per second or more were invariably associated with the presence of histologically recognizable thyroid in the graft at the end of the experiment, and there was a rough correlation between the volume of this tissue and the 24-hr. count in the last iodine uptake test; accurate comparison was difficult, however, owing principally to the fact that each graft consisted of many small pieces of tissue.

When the 24-hr. count was followed by two or more later counts the values obtained were plotted against time on semilogarithmic paper, and, in about half the tests with autografts and three-quarters of the tests with homografts, the points were found to lie approximately on a straight line. It would seem therefore that from the twenty-fourth hour onward the ^{131}I content of the grafts often decreased exponentially with time, though with only three or at most four points to define each line this conclusion must be accepted with

TABLE III.—UPTAKE OF ^{131}I BY THYROID AUTOGRAFTS

Animal No.	No. of test	Weeks after grafting	Counts per second over graft Hours after I^{131} injection					Post mortem examination of neck
			8	24	48	72	96	
326	First	6	—	92·8	55·7	22·4	39·7	Nodule of regenerating thyroid
	Second	10	68·4	64·3	68·3	29·0	24·4	
	Third	16	30·9	35·5	13·8	7·1	—	
329	First	6	34·3	57·2	36·5	7·0	2·9	
	Second	10	37·9	35·6	9·7	15·3	12·6	
	Third	16	33·5	35·6	23·7	11·1	—	
330	First	6	76·4	137·3	100·4		52·3	
	Second	10	43·0	48·0	30·3	28·8	40·2	
	Third	16	53·0	44·1	26·7	11·5	—	
332	First	6	—	88·1	68·5	45·9	36·3	
	Second	10	24·5	27·3	18·9	15·3	10·2	
	Third	16	29·8	32·9	27·3	8·3	—	
679	First	6	41·2	51·9	35·8	23·0	—	
	Second	10	34·6	29·3	18·6	11·6	—	
	Third	16	—	70·9	43·3	29·0	—	
680	First	6	75·2	67·4	38·6	19·0	—	
	Second	10	72·4	81·0	62·4	26·9	—	
	Third	16	—	68·9	72·1	40·3	—	
681	First	6	117·5	130·8	101·8	82·5	—	
	Second	10	66·7	76·5	66·6	53·2	—	
	Third	16	—	75·2	65·1	46·7	—	
684	First	6	74·9	72·6	52·3	60·6	—	
	Second	10	55·5	70·1	51·6	52·8	—	
	Third	16	—	96·0	102·6	65·6	—	
685	First	6	79·8	55·5	40·0	19·9	—	
	Second	10	53·3	44·1	29·9	19·9	—	
	Third	16	—	86·0	59·9	31·6	—	
686	First	6	51·1	54·2	60·1	44·4	—	
	Second	10	62·2	65·1	56·3	40·3	—	
	Third	16	—	104·4	76·9	66·2	—	

— Denotes count omitted owing to counter not being available.

caution. The departures from linearity may indicate some genuine exceptions to this rule but they may on the other hand be due to erroneously low counts resulting from failure to centre the animal accurately over the counter.

TABLE IV.—UPTAKE OF ^{131}I BY THYROID HOMOGRAFTS IN HOSTS INJECTED AT BIRTH WITH DONOR CELLS

Host No.	No. of test	Weeks after grafting	Counts per second over graft Hours after ^{131}I injection			Post mortem examination of neck
			24	48	72	
427	First	6	41·7	23·5	11·3	
	Second	10	61·7	41·3	29·9	
	Third	16	74·7	—	—	
430	First	6	59·0	32·0	35·8	
	Second	10	59·8	45·1	38·4	
	Third	16	105·5	—	—	
439	First	6	1·2*			Nodule of regenerating thyroid
	Second	10				
	Third	16				
442	First	6	67·5	39·6	27·5	
	Second	10	72·9	46·9	30·5	
	Third	16	104·6	—	—	
445	First	6	75·9	44·7	39·4	
	Second	10	61·0	38·2	26·2	
	Third	16	63·9	—	—	
451	First	6	87·1	49·8	49·3	
	Second	10	88·0	65·5	45·8	
	Third	16	96·2	—	—	
454	First	6	46·1	27·7	20·6	
	Second	10	77·9	44·6	32·9	
	Third	16	80·3	—	—	
457	First	6	49·3	38·2	24·6	
	Second	10	72·3	42·7	25·1	
	Third	16	66·5	—	—	
463	First	6	44·9	24·4	17·1	
	Second	10	71·0	51·5	27·7	
	Third	16	67·0	—	—	

* Animal killed after this count. No thyroid tissue found at site of graft.
— Denotes count omitted owing to counter not being available.

If the law of decay is expressed in the form $C_t = C_1 e^{-\lambda(t-1)}$, where C_t denotes the count t days after ^{131}I injection, C_1 denotes the count 1 day (24 hr.) after ^{131}I injection, and h denotes the effective half-life of ^{131}I in the graft during the phase of exponential decay in days, then the slope of the line in the semi-logarithmic plot is proportional to λ, and $h = \frac{\log_e 2}{-\lambda}\cdot$ The values of $-\lambda$ and h have been calculated for all cases in which a linear plot was obtained and the values are shown in figs. 3 and 4. Brown-Grant, von Euler, Harris and Reichlin [1954] have produced convincing evidence that the slope of the ^{131}I release curve provides a good index of the activity of the intact thyroid in the rabbit, and it seems likely that the same is true of the activity of the thyroid grafts in this and other species including the rat.

In vivo counts at the normal site of the thyroid gland 24 hr. after ^{131}I injection were usually less than eight counts per second, but in four animals Nos. 326, 439, 446, 458) were in the range twelve to twenty-one counts per second. In these four animals, but in no others, a nodule of regenerating thyroid was found in the neck. When, however, the larynx and related structures were removed *en bloc* from five of the amimals in which there did not appear to be any regenerating thyroid and tested with a Geiger-Müller counter, β emission considerably in excess of that obtained from a block of tissue of the same size from elsewhere in the animal was detected. It appears therefore that some thyroid cells had been left behind even in these animals, and it seems likely that if the same test had been performed in other animals the results would have been the same.

TABLE V.—UPTAKE OF ^{131}I BY THYROID HOMOGRAFTS IN CONTROL HOSTS

Host No.	No. of test	Weeks after grafting	Counts per second over graft Hours after grafting 24	48	72	Post mortem examination of neck
428	First	6	6·3*			
431	First	6	2·5*			
440	First	6	25·5	11·5	13·9	
	Second	10	21·3	17·1	13·9	
	Third	16	15·2	—	—	
443	First	6	1·8*			
446	First	6	2·1*			Nodule of regenerating thyroid
452	First	6	1·4*			
455	First	6	1·3*			
458	First	6	6·3*			Nodule of regenerating thyroid
464	First	6	0·4*			

* Animal killed after this count. No thyroid tissue found at site of graft.
— Denotes count omitted owing to counter not being available.

It seems safe to ignore scattered thyroid cells invisible to the naked eye, but a visible nodule of regenerating host thyroid tissue, though associated with a functioning graft in one animal (No. 326), may to some extent have prejudiced graft survival in the others [*c.f.* Woodruff and Woodruff, 1950].

Comparison of Autografts and Homografts.—The autografts, and eight of the nine homografts in hosts previously injected with donor cells, behaved indistinguishably. The range of variation in iodine uptake (Table VI) and in functional activity (figs. 3 and 4) were virtually the same in the two groups. Histological examination after the grafts had been in place for 16 weeks also revealed no difference, and in particular no evidence of host reaction in or around the homografts (figs. 5 and 6).

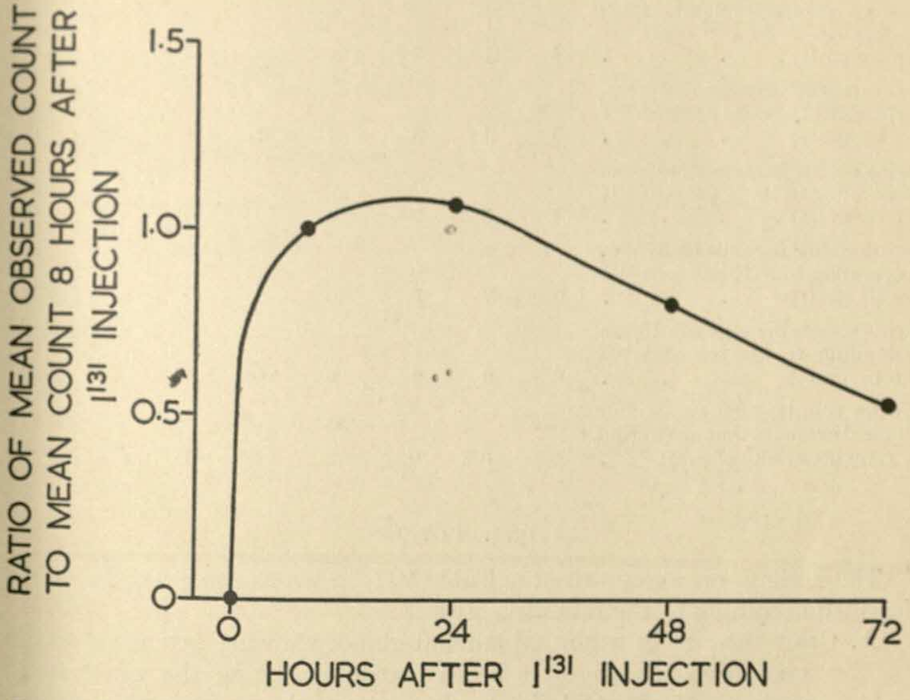

FIG. 2.—Graph showing the mean ratio of the count over the graft at various intervals after injection of ${}^{131}I$ to the count 8 hr. after injection for twenty tests with thyroid autografts.

On the other hand eight of the nine homografts in control hosts were destroyed within 6 weeks. The uptake of iodine was negligible and histological examination at this stage revealed either no trace of the graft or a mass of connective tissue densely infiltrated with small round cells and containing no recognizable thyroid epithelium (fig. 7).

In two animals the grafts behaved anomalously. One (No. 439) had been injected with donor cells at birth but in spite of this the graft was destroyed within 6 weeks. The other (No. 440) was a control, and thyroid tissue survived throughout the experiment; the iodine uptake was always relatively low however and histological examination after 16 weeks revealed a very small piece of thyroid surrounded by dense connective tissue.

Table VI.—Uptake of Iodine by Thyroid Grafts. Based on Counts made 24 hr. after Intraperitoneal Injection 5 μc ^{131}I

	Autografts			Homografts to hosts injected at birth with donor cells			Homografts to control hosts		
	6 weeks	10 weeks	16 weeks	6 weeks	10 weeks	16 weeks	6 weeks	10 weeks	16 weeks
No. of animals in group . . .	10	10	10	9	9	9	9	9	9
No. showing counts > 80/sec. (corresponding to > 30 per cent ^{131}I uptake by graft)	4	1	3	1	1	4	0	0	0
No. showing counts in range 53–80/sec. (corresponding to 20–30 per cent ^{131}I uptake by graft)	5	4	3	3	7	4	0	0	0
No. showing counts in range 27–53/sec. (corresponding to 10–20 per cent ^{131}I uptake by graft)	1	3	3	4	0	0	0	0	0
No. showing counts in range 13–27/sec. (corresponding to 5–10 per cent ^{131}I uptake by graft)	0	2	1	0	0	0	1	1	1
No. showing counts in range 6·5–13/sec. corresponding to 2–5 per cent ^{131}I uptake by graft)	0	0	0	0	0	0	0	0	0
No. showing counts < 6·5 sec. and/or histological evidence that no thyroid tissue remains at site of graft .	0	0	0	1	1	1	8	8	8

Adrenal Grafts

The findings are summarized in Table VII, in which the grafts have been classified according to the following criteria:

A. Graft the size of a normal adrenal gland, showing extensive cortical regeneration with three zones corresponding to the zones in the cortex of the normal gland. In many of the grafts in this category there was a central area of necrotic material or fibrous tissue which as a rule showed patchy calcification, but elsewhere there was no increase in connective tissue and there was no infiltration with "lymphocytes".*

B. Graft the size of a normal adrenal gland or a little smaller, showing islets of regenerating cortical tissue without clearly defined zones, separated by septa of connective tissue with little or no "lymphocyte" infiltration.

C. Graft less than half the diameter of a normal adrenal gland, showing a marked "lymphocytic" or fibroblastic reaction but containing recognizable cortical tissue.

D. No recognizable cortical tissue found at site of graft.

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* No attempt has been made to distinguish between lymphocytes and plasma cells, and the term "lymphocyte" is used to include both.

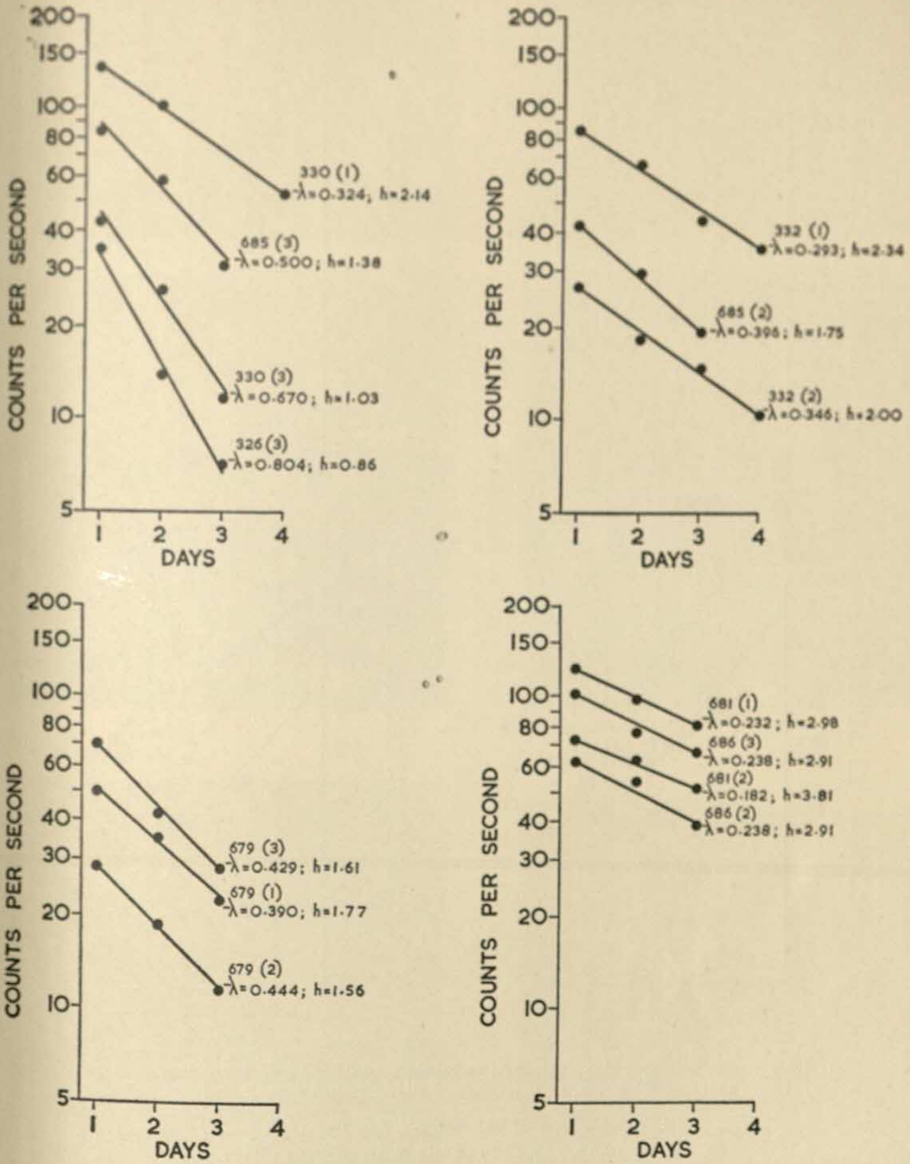

Fig. 3.—Graphs showing the change in radioactivity of thyroid autografts starting 24 hr. after injection of ^{131}I plotted semi-logarithmically.

$-\lambda$ = proportion of ^{131}I lost per day from the graft.
h = effective half life of ^{131}I in the graft in days.

Tests in which the graph was not approximately linear have been excluded.

Autografts.—The six animals which received autografts all remained in good health for the 16 weeks during which one or both grafts were in place. The three grafts removed after 4 weeks and the nine grafts removed after 16 weeks were all of type A (fig. 8).

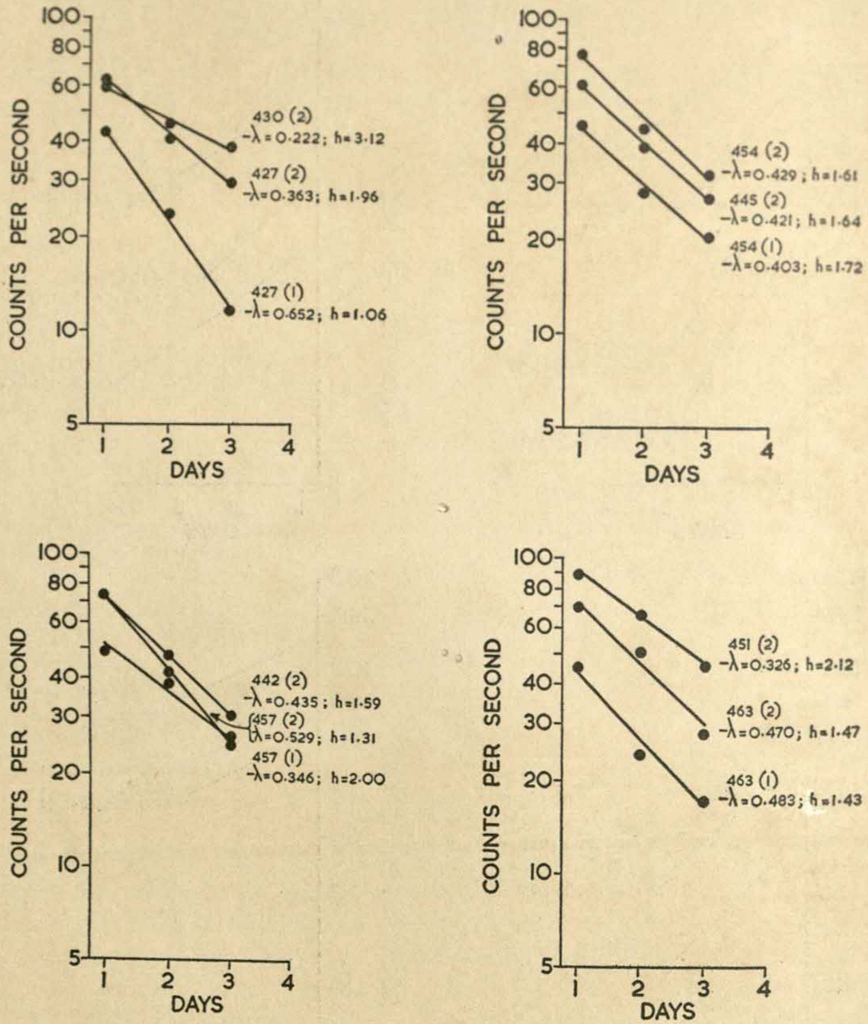

FIG. 4.—Graphs showing the change in radioactivity of thyroid homografts in tolerant hosts starting 24 hr. after injection of ^{131}I, plotted semi-logarithmically.

$-\lambda$ = proportion of ^{131}I lost per day from the graft.
h = effective half life of ^{131}I in the graft in days.

Tests in which the graph was not approximately linear have been excluded.

FIG. 5.—Thyroid autograft after 16 weeks. H. and E. ×125.

FIG. 6.—Thyroid homograft in tolerant host after 16 weeks. H. and E. ×125.

FIG. 7—Thyroid homograft in control host after 6 weeks, showing gross "lymphocytic" infiltration. H. and E. ×125.

FIG. 8.—Adrenal autograft after 16 weeks showing good cortical regeneration with three well-defined zones. There is a small central scar showing patchy calcification. Histological type A. H. and E. ×100.

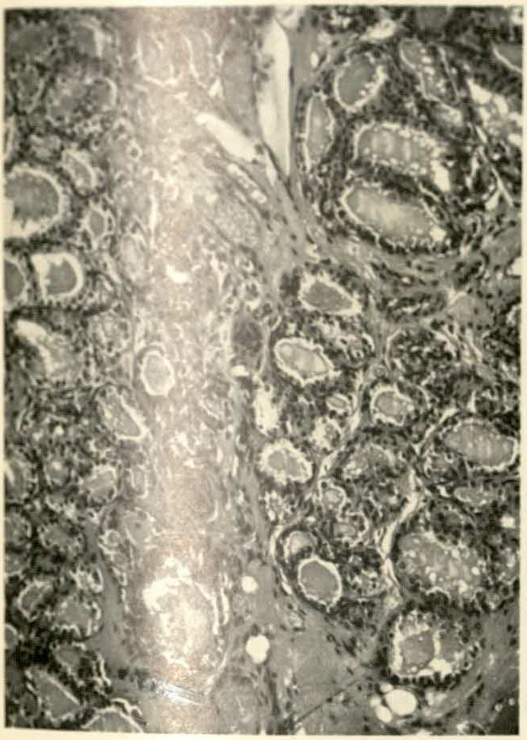

FIG. 5

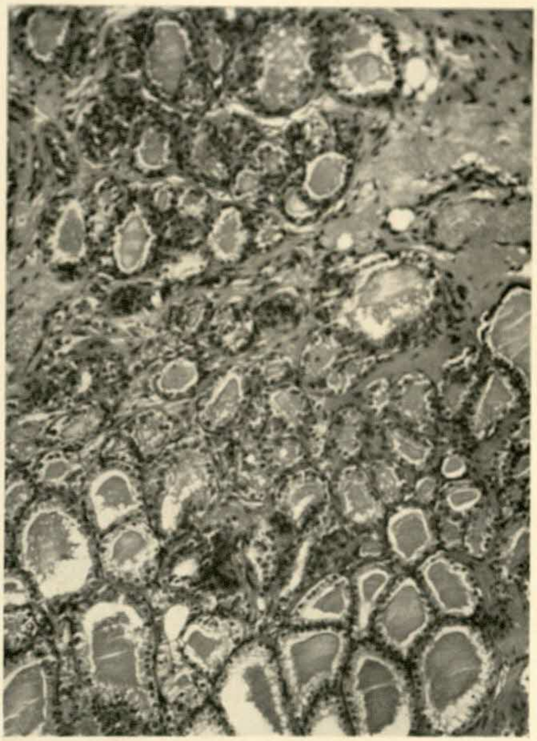

FIG. 6

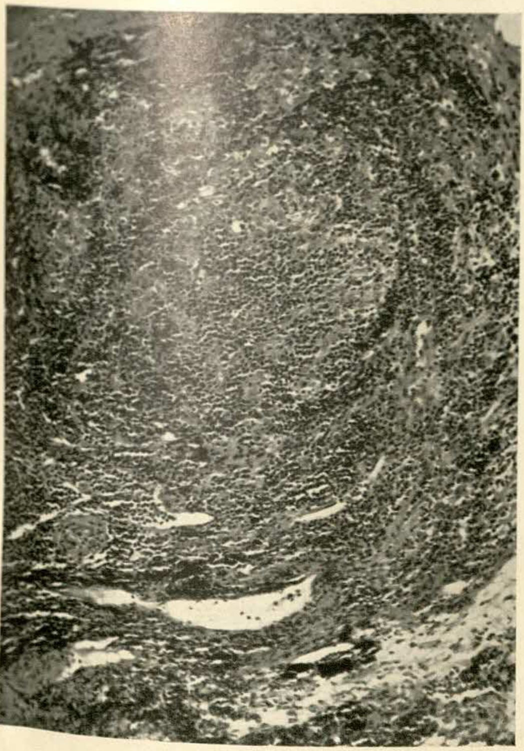

FIG. 7

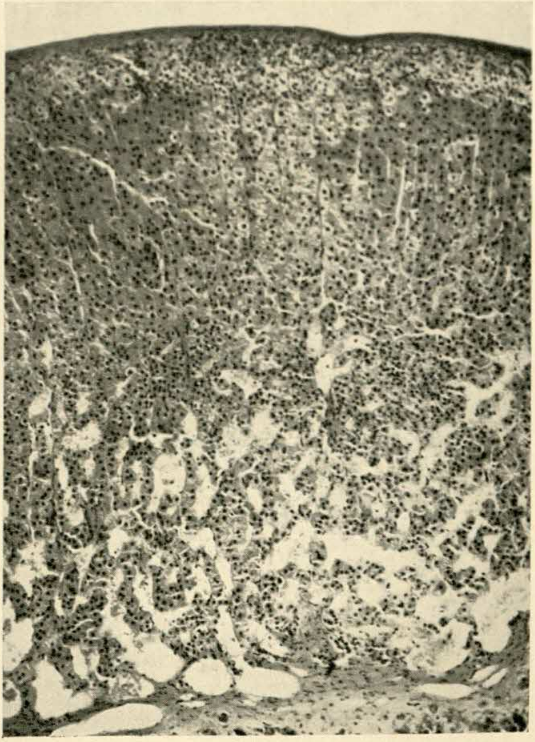

FIG. 8

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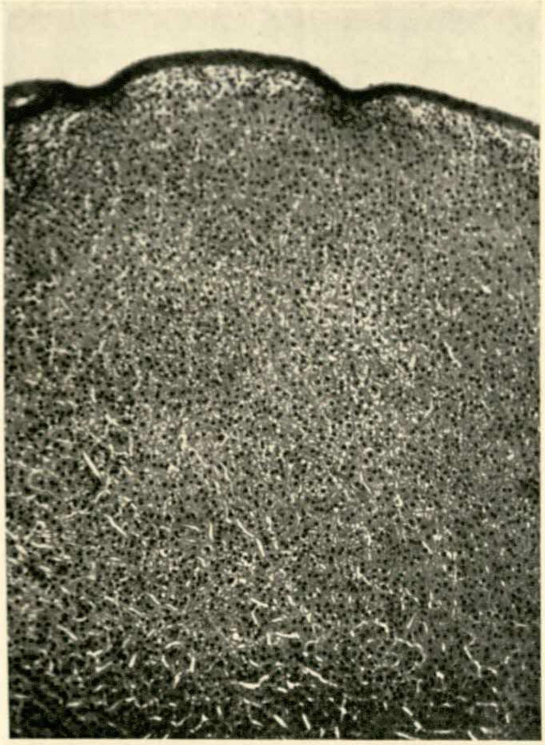
FIG. 9

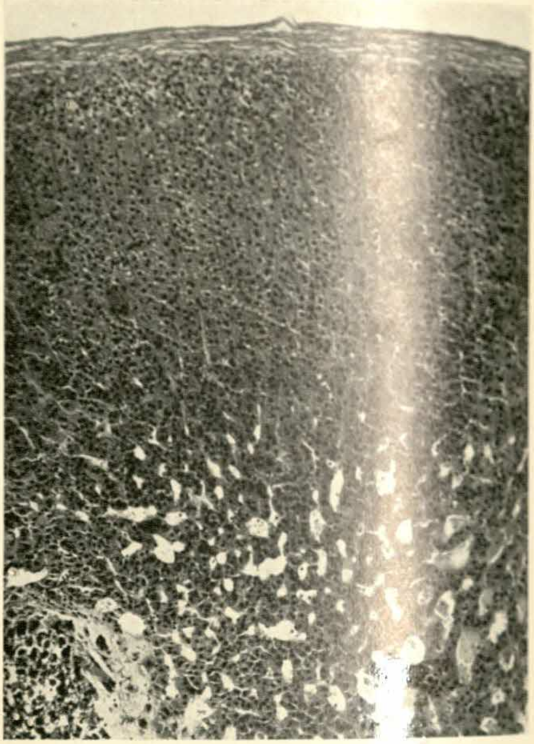
FIG. 10

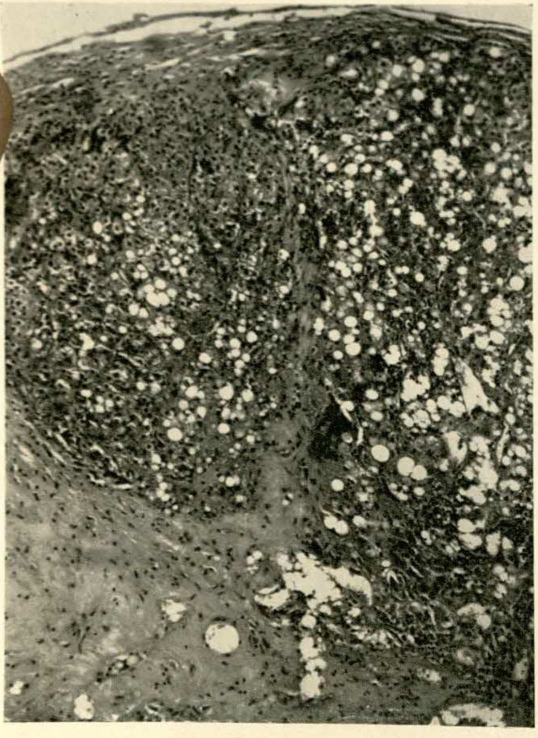
FIG. 11

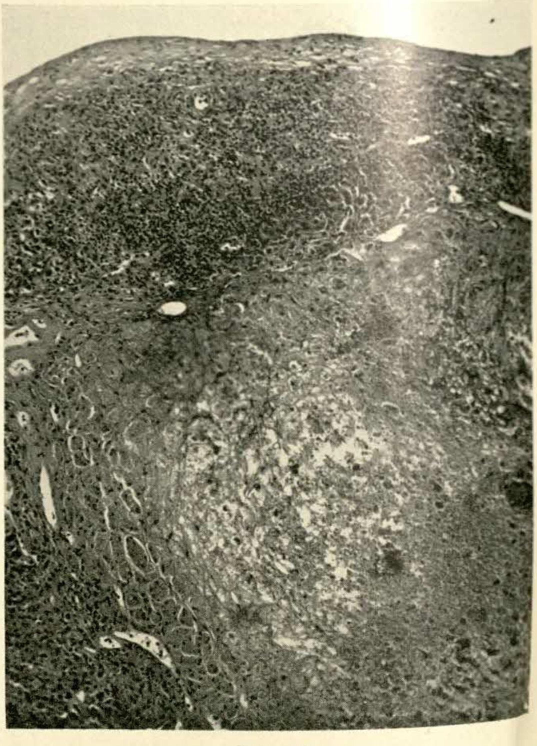
FIG. 12

Homografts in Hosts Injected at Birth with Donor Cells.—Six of the seven animals which had been injected at birth with donor cells remained in good health for the 16 weeks during which one or both grafts were in place, and all except one died within 6 weeks after removal of the second graft. All the grafts in these animals were of type A (fig. 9).

TABLE VII.—ADRENAL GRAFTS

Graft and host	No. of animals in group	Animals surviving less than 16 weeks after grafting			Animals surviving more than 16 weeks after grafting		
		No.	Period of survival weeks	Histological classification* of grafts	No.	Histological classification * of grafts removed after 4 weeks (autografts) or 8 weeks (homografts)	Histological classification* of grafts removed after 16 weeks
Autografts .	6	0			6	AAA	AAAAAA
Homografts in hosts injected at birth with donor cells .	7	1	5	C	6	AAAAAA	AAAAAA
Homografts in control hosts	7	3	5, 6, 6	C, C, C	4	AAAC	ABBB

* The criteria used are described in the text.

One cell-injected animal died after 5 weeks and the grafts removed immediately after death were of type C.

Homografts in Control Hosts.—Four of the seven control animals remained in good health for the 16 weeks during which the grafts were in place, and two of these died within 6 weeks after removal of the second graft. Three of the grafts removed after 8 weeks were of type A, whereas of the grafts removed after 16 weeks only one was of type A (fig. 10) and three were of type B (fig. 11).

The other three animals died 5–6 weeks after grafting, and their grafts removed immediately after death were of type C (fig. 12).

CONCLUSIONS AND DISCUSSION

Observations on the uptake and release of ^{131}I appear to provide a reliable means of assessing the behaviour of thyroid grafts, and in particular the amount of surviving thyroid tissue and its level of functional activity.

FIG. 9.—Adrenal homograft in host injected at birth with donor cells, showing good cortical regeneration with three well-defined zones. Histological type A. H. and E. ×100.

FIG. 10.—Adrenal homograft after 16 weeks in control host. Histological type A. H. and E. ×100.

FIG. 11.—Adrenal homograft after 16 weeks in control host. Histological type B. H. and E. ×100.

FIG. 12.—Adrenal homograft after 6 weeks in control host. Histological type C. H. and E. ×100.

Homografts of thyroid, placed alongside the femoral vessels in thyroidectomized rats which received at birth a subcutaneous injection of spleen cells from the same donor, are indistinguishable from autografts both in their capacity to take up radioactive iodine and in their histological structure. Homografts in thyroidectomized but otherwise untreated hosts, on the other hand, are usually completely destroyed within 6 weeks. It seems clear therefore that subcutaneous injection of homologous spleen cells at birth normally results in a high degree of tolerance to thyroid tissue from the same donor.

Homografts of whole adrenal glands sutured to the ovaries of adrenalectomized hosts which received at birth a subcutaneous injection of donor spleen cells also behave like autografts; *i.e.* the cortex regenerates and functions, while the medulla disappears and is replaced as a rule by connective tissue containing deposits of calcium. This suggests that injection of spleen cells results also in tolerance to adrenal cortical tissue. The evidence, however, is not quite as conclusive as in the case of thyroid grafts because, as reported previously [Woodruff and Boswell, 1953] and confirmed in the present experiments, adrenal homografts to the ovary sometimes survive for several months even in control hosts. Such grafts however sooner or later show evidence that some of the cortical tissue is being replaced by connective tissue, and it seems likely that if the grafts were left in place this process would continue until they were completely destroyed.

The reason for this long survival of a proportion of adrenal homografts in control hosts has not been elucidated. Neither the hooded rats used as donors nor the albinos used as hosts were drawn from genetically uniform populations, but they had been propagated for many years by mating within each colony and it seems likely that the antigenic differences between members of different colonies were much greater than those between members of the same colony. Moreover, the survival of skin homografts from hooded rats to albinos, using members of the same two colonies, has been found in several hundred trials to be remarkably constant and has never exceeded 18 days [Woodruff and Simpson, 1955 *a*]. It seems unlikely, therefore, that fortuitous antigenic similarity of donor and host can account for the long survival of adrenal grafts in some of the control hosts, though it may have played some part. The question arises therefore as to whether the long survival is due, or partly due, to some special property of adrenal cortical tissue or, as seems perhaps more likely, of the site in which the transplants were located. It is planned to study the problem further using two inbred strains of rats which have recently become available to one of us.

The discovery that tolerance induced by spleen cells is not confined to skin homografts but extends also to homografts of endocrine tissue provides strong evidence that all the transplantation antigens present in the cells which constitute these tissues are present in the cells of the spleen, and is consistent with the view that the antigens concerned in transplantation immunity are common to all the nucleated cells of an individual. To clinch the matter it would be necessary to show that a homotransplant of any one tissue to a sufficiently immature animal induced tolerance to homotransplants of all other tissues from the same donor. This presents technical difficulties because it is difficult to transplant solid pieces of tissue to embryos *in utero*, and transplantation of skin at birth—even in the rat which would seem to be the most favourable of the usual laboratory animals [*c.f.* Woodruff and Simpson, 1955 *b*]—has so far not proved to be a very efficient method of inducing tolerance. It has been found however that skin grafting at birth induces partial tolerance, and occasionally apparently complete tolerance, to a subsequent graft of skin [Medawar and Woodruff, 1958] or thyroid [Woodruff and Sparrow, unpublished] from the same donor.

ACKNOWLEDGMENTS

We are indebted to Dr. H. D. Purves, Director of the Endocrinology Research Unit of the New Zealand Medical Research Council, for the loan of the Scintillation counter, and to Dr. D. Adams for much helpful advice regarding the ^{131}I uptake tests.

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ERYTHROPOIESIS: AN ELECTRON MICROSCOPICAL STUDY.

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Embryonic blood from rats and rabbits, and human blood and bone marrow have been examined in the electron microscope after osmium fixation and methacrylate embedding. The dense cytoplasm of the mature erythrocyte absorbs electrons, a feature which is probably due to the presence of hæmoglobin. The acquisition of this property identifies the maturing erythroblasts. The increase in density of the cytoplasm during hæmoglobin formation is matched by a similar change in the nucleoplasm. It is considered that this is due to the presence of hæmoglobin or closely associated iron containing precursors in the nucleus. During maturation the nucleo-cytoplasmic boundary gradually disappears. This observation opposes the view that the nucleus is extruded as a whole. In the early erythroblast, ribonucleic acid granules are present in the cytoplasm. These are not evident in the later stages of maturation and at no time is a well developed endoplasmic reticulum present.

THE introduction of thin sectioning techniques [Porter and Blum, 1953; Newman, Borysko and Swerdlow, 1949] makes it possible to study the fine structure of blood cells and blood forming tissues. Most of the published observations [Braunsteiner, Fellinger and Pakesch, 1957; Goodman, Reilly and Moore, 1957; Grey and Biesele, 1955; Rinehart, 1955] deal with normal and leukæmic white blood cells. Isolated observations on developing red blood cells are made by a number of authors [Bernhard and Leplus, 1955; Bessis, 1956; Braunsteiner, Fellinger and Pakesch, 1956; Kautz and De Marsh, 1955] and a careful study of the guinea-pig bone marrow is given by Pease [1956].

The results of an electron microscopic study of erythropoiesis in mammalian embryos and adult human bone marrow, after fixation and embedding by the usual techniques, are reported in this paper.

MATERIAL AND METHODS

Collection of Specimens and Fixation. Embryos.—At known periods after coitus, embryos were removed rapidly from rats and rabbits killed with ether. The younger embryos were fixed whole, but the older ones were cut into portions of less than 2 cu. mm. before fixation for 1 hr. The fixatives employed were 1 per cent osmium tetroxide buffered with chromate [Dalton, 1955] formol saline and Bouin's fluid.

Bone Marrow.—The following technique for preparing marrow sections was the most successful in our experience. Human marrow was obtained by sternal puncture with a dry siliconed syringe and needle. Several drops of marrow aspirate were immediately transferred to a tube containing Dalton's fixative. The remainder was mixed with 3·8 per cent sodium citrate for preparing marrow smears. The fixative was allowed to stand for 5 min., by which time the heavier marrow particles formed a sediment while the marrow blood remained in suspension. The supernatant fluid was poured off and the marrow particles were resuspended in fresh fixative for a further hour. Other specimens were fixed in Zenker's fluid, formol saline and methyl alcohol.

Circulating Blood.—Human blood was obtained by venepuncture into a siliconed syringe and transferred to a siliconed test tube, which was then spun at 2000 r.p.m. in a standard hæmatocrit centrifuge for 10 min. The "buffy coat" was pipetted off with a siliconed Pasteur pipette and transferred to Dalton's fixative for 1 hr.

Dehydration and Embedding.—All specimens were dehydrated through graded alcohols and infiltrated with a mixture of methyl and butyl methacrylate monomers in the proportion of 1:9. Blood and small marrow particles were centrifuged gently at under 1000 r.p.m. for about 2 min. between changes of alcohol and methacrylate. Faster or more prolonged spinning packed the cells into a solid mass which it was difficult to resuspend. To permit separation of the cells within 2 min., a short column of fluid (not more than 2 cm.) must be employed in the centrifuge tube. The methacrylate mixture, with benzoyl peroxide as an initiator, was incubated at 56° C. in 00 gelatin capsules until it had a syrupy consistency. Tissues were then added and polymerization was completed by incubation for 12 hr. at 37° C.

Sectioning and Examination of Tissues.—Sections were cut for electron microscopy at 200 Angstrom units (Å) on a Porter-Blum [1953] microtome equipped with a glass knife. These sections were examined in a Metropolitan Vickers EM 3 microscope with a 50 μ objective lens aperture. The initial magnifications were between 700 and 5000, with subsequent photographic enlargement.

In parallel with electron microscopy we examined 1 μ sections of methacrylate embedded tissue by phase contrast microscopy and by several staining methods. After osmium fixation, Romanowsky staining did not differentiate the cells as cytoplasmic acidophilia was lost. Consequently it was not possible to identify all the cells in the electron micrograph by relating them, cell by cell, to a stained adjacent section.

Smears of blood and bone marrow taken from the original specimens were examined after Romanowsky staining.

Nomenclature.—The term "erythrocyte" is used throughout this article to describe the mature red blood cell, and "erythroblast" to describe any nucleated red cells, whether embryonic or adult. The more mature adult erythroblasts are referred to as "normoblasts".

OBSERVATIONS

After osmium fixation, the mature erythrocyte is easily identified in the electron micrograph by its intense and uniform electron absorption. Only erythrocytes possess this property and its acquisition can therefore be used to identify erythroblasts. The cells thus identified have morphological characteristics, such as agranular cytoplasm and round central nuclei which correspond to those of erythroblasts as observed with the light microscope, and the proportion of marrow cells identified by this method as erythroblasts corresponds to the number seen in stained smears made from the same specimens.

The increase in electron absorption which characterizes the erythroblast occurs during the period in which hæmoglobin is deposited and this progressive increase is considered to be related to hæmoglobin formation.

Embryonic Erythropoiesis.—The embryonic erythroblast (fig. 1) is a large roughly spherical cell, bounded by a smooth definite cell membrane.

Throughout the cytoplasm there are very many small dense spherical particles (P), similar to those which Palade [1955] describes and considers to be the cytoplasmic ribonucleic acid granules. There is no well developed endoplasmic reticulum and these particles are scattered at random throughout the cytoplasm. In one section (fig. 1) a group of vesicles and closely packed lamellæ (G) is seen in the cytoplasm. On the basis of its similarity to the structure described by Dalton and Felix [1953, 1954] and Lacy and Challice [1956] this structure is identified as the Golgi apparatus. The mitochondria (M) are round or oval in section and have the relatively simple system of internal membranes which is stated to be typical of embryonic cells [Dempsey, 1956].

The large spherical nucleus (fig. 1) is limited by a clearly defined nuclear membrane. The prominent nucleoli consist of closely packed dense granules arranged in a coarse open network. The remainder of the nucleoplasm, which has a fine granularity, is made up of alternate areas of moderate and low electron absorption.

Many of these features are retained by the more mature embryonic erythroblast (figs. 2 and 3), but the deposition of hæmoglobin or associated iron containing compounds is shown as an overall increase in the density of the cell; the cytoplasm now has a uniform ground-glass appearance and the contained mitochondria are seen as clear spaces. The formerly clear areas in the nucleoplasm now possess almost the same density and texture as the cytoplasm. In contrast to light microscopy specimens, the thinness of these sections (200 Å) makes it certain that this nuclear density is not due to superimposed cytoplasm. The absence of endoplasmic reticulum in the cytoplasm of these cells is made more conspicuous by its demonstration in neighbouring mesenchymal cells (fig. 2). Embryonic blood contains erythroblasts with varying degrees of hæmoglobin formation, but in all the more mature cells the nucleoplasm shows evidence of hæmoglobin deposition which is only slightly less advanced than in the cytoplasm.

Adult Erythropoiesis.—A low power electron micrograph of adult human bone marrow (fig. 5) shows the steady increase in density of the more mature

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FIG. 1.—Rat embryo obtained 10½ days after coitus; Dalton fixation. Fœtal erythroblast from the circulating blood. × 14,000.

The nucleus in the left upper quadrant is bounded by a clearly defined nuclear membrane. A large, reticular, electron-dense nucleolus is surrounded by nucleoplasm which is organized into moderately dense and clear areas.

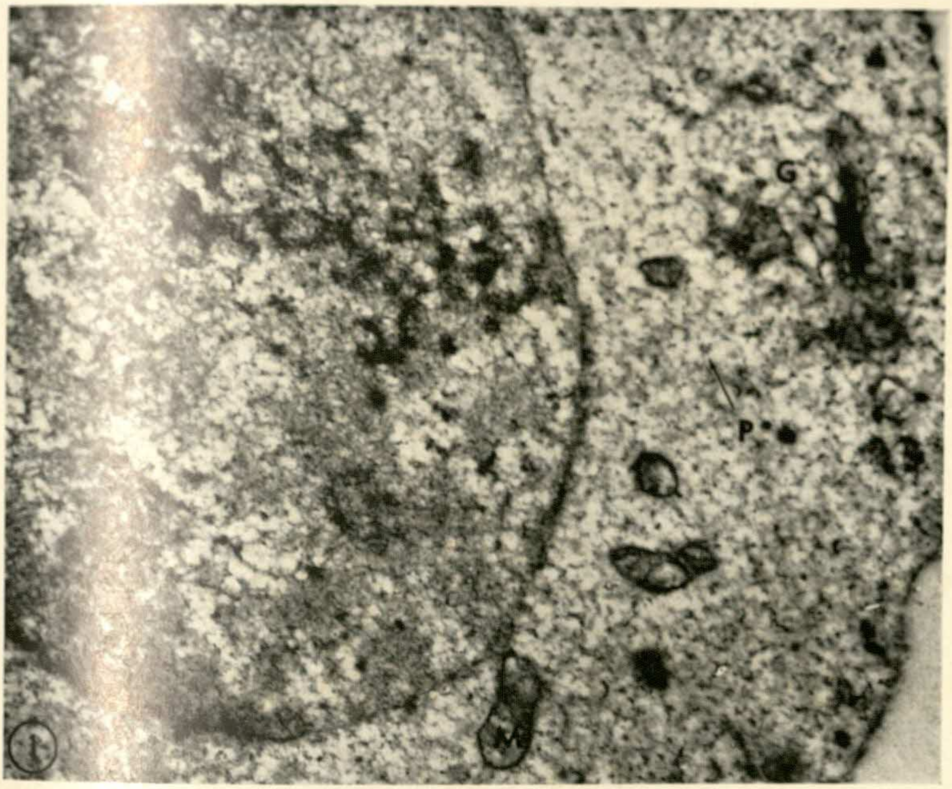
Mitochondria (M) and the Golgi apparatus (G) are shown within the cytoplasm, which does not contain a well developed endoplasmic reticulum. Scattered throughout the cytoplasm there are large numbers of dense spherical particles (P) which have a diameter of approximately 200 Å.

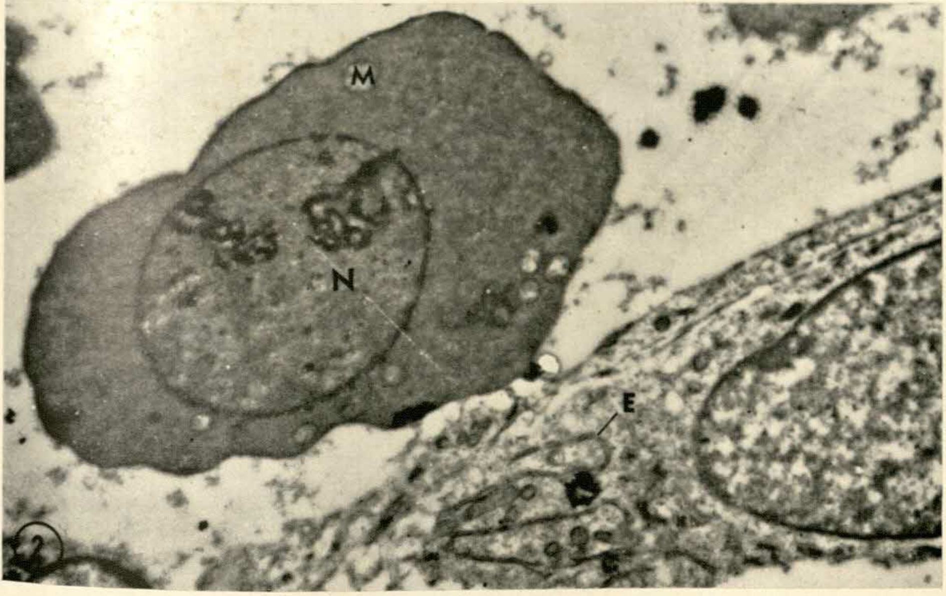
FIG. 2.—Rabbit embryo obtained 10 days after coitus. Dalton fixation. Nucleated red blood cell against the wall of a blood vessel. × 6000.

The nucleoplasm (N) and the cytoplasm of the red blood cell have a greater electron density than those of the adjoining mesenchymal cell.

Mitochondria (M) are evident as clear areas in the cytoplasm which is otherwise homogeneous. Endoplasmic reticulun (E) which is well developed in the mesenchymal cell is absent in the blood cell.

As in Fig. 1, the nuclear membrane and nucleoli are easily distinguished, but the overall density of the nucleoplasm has increased and approaches that of the cytoplasm.





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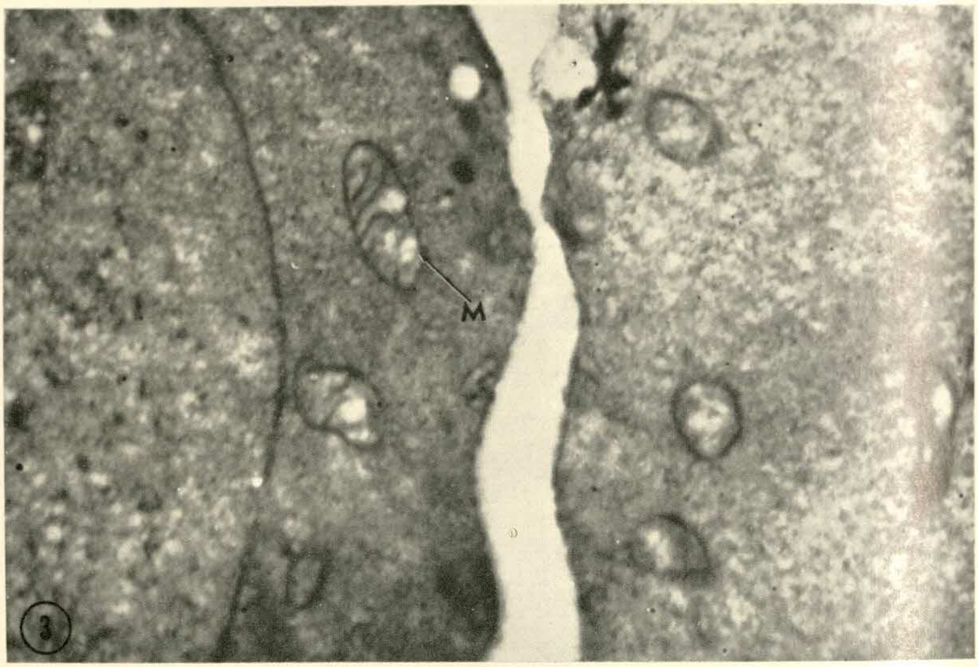
③
M

④
A
B
C
D
E
M

erythroblasts. Their identification is confirmed by the presence of granules, endoplasmic reticulum and lobed nuclei in many of the developing white cells (WBC 2). A small proportion of the marrow cells have no distinguishing features which assign them to red or white cell series (WBC 1). They have large nuclei with nucleoli and are thought to be the early erythroblasts, myeloblasts and stem cells.

As in the embryonic erythroblast, the nucleus of the adult erythroblast (EB, fig. 5) is characterized by its large size, prominent nucleoli and well defined nuclear membrane. Mitochondria, which are present in the cytoplasm at all stages of development of the erythroblast are mostly circular in section though some are elongated suggesting a rod-like structure. In the later stages their internal matrix acquires a density similar to that of the surrounding cytoplasm, a feature which is not observed in the embryonic erythroblast. A well developed endoplasmic reticulum is not seen in any of the red cell series.

During maturation of the erythroblast, the increase in electron absorption associated with hæmoglobin formation affects the nucleus in the same manner, and at the same rate, as the cytoplasm. In the earlier part of this process the nucleus retains its nucleoli, spherical shape, large size and prominent limiting membrane. In the later stages the cell and its nucleus decrease in size and there is a marked increase in density of both. At the same time the nucleo-cytoplasmic boundary becomes less and less distinct until it can no longer be identified, though in some specimens an artificial separation of nucleus and cytoplasm makes evident an otherwise indistinguishable nucleus (N fig. 5). In some of the late normoblasts (figs. 5 and 8) aggregations of nuclear material are seen which are denser than either the cytoplasm or the surrounding nucleoplasm. They usually lie in relation to the nuclear membrane. At this stage lobulation of the nucleus is often obvious (fig. 8) and in the most mature normoblasts either fragmentation or extreme lobulation is often seen.

Preparations consisting largely of normoblasts and mature white cells (which are easily distinguished) can be conveniently prepared by centrifugation of circulating blood from patients with myelofibrosis. In this rare condition fibrosis of the bone marrow is associated with extramedullary

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FIG. 3.—Rabbit embryo obtained 10 days after coitus. Dalton fixation. Portions of two nucleated red blood cells. × 25,000.

The characteristic internal structure of mitochondria is seen. In the right hand cell a distinct nuclear membrane separates a dense and uniform cytoplasm from the nucleoplasm which has a similar texture and only slightly lower density. The cytoplasm of a less mature cell is seen on the left.

FIG. 4.—Segments of electron micrographs of developing red blood cells (A and B embryonic; C, D and E adult human). Dalton fixation. × 16,000. Mounted side by side with the nucleus above.

In the least mature cell (A) the nucleus is denser than the cytoplasm, a relationship which is reversed in (B) while with increasing maturity (C, D and E) the differences between nucleus and cytoplasm diminish. This change is accompanied by a decrease in prominence of the nucleo-cytoplasmic boundary (arrows). This boundary can only be distinguished with difficulty in (E) although adjacent mitochondrial membranes (M) are clearly seen.

erythropoiesis which takes place in liver and spleen; red cell precursors are released into the circulation at an earlier stage of development than usual. Because of the high proportion of almost mature normoblasts found in these specimens a favourable material is provided for the study of the last stages of the disappearance of the nucleo-cytoplasmic boundary. In fig. 7 the boundary can just be discerned, but in many electron micrographs it is impossible to decide whether a membrane is present or not. As it is absent in the mature erythrocyte we conclude that the boundary disappears at a late stage in maturation. This process is illustrated in fig. 4 in which electron micrographs of segments of cells at different stages of maturation are mounted side by side.

Erythrocytes are very liable to be compressed during sectioning. The nuclei of white cells and early erythroblasts do not usually show compression even when neighbouring erythrocytes are severely corrugated. As the nucleus of the late normoblast is compressed as easily as its cytoplasm, it appears that the nucleoplasm resembles the cytoplasm in consistency as well as in electron absorption.

After osmium-chromate fixation, the electron absorption by a cell may be due either to the combination of these heavy metals with the cell or to its inherent density. In order to assess inherent density without this complication, bone marrow is fixed in formol-saline (fig. 9). Although much of the fine structure in electron micrographs is lost, the nuclear membrane is clearly shown and in such specimens the nucleus and cytoplasm of the erythroblast show an even more striking similarity in texture and density than in osmium fixed tissue. The increase in density of the erythroblast nucleus during maturation cannot therefore be explained as an increase in osmophilia of the nucleus that keeps pace with the deposition of hæmoglobin in the cytoplasm. After formol-saline fixation the electron absorption by cells known to contain hæmoglobin is greater than that of the white cells and we therefore believe that hæmoglobin itself is responsible for the density of these cells.

Fig. 5.—Adult human bone marrow, showing active normoblastic erythropoiesis 10 days after commencing treatment for Addisonian megaloblastic anæmia. Dalton fixation. × 3000.

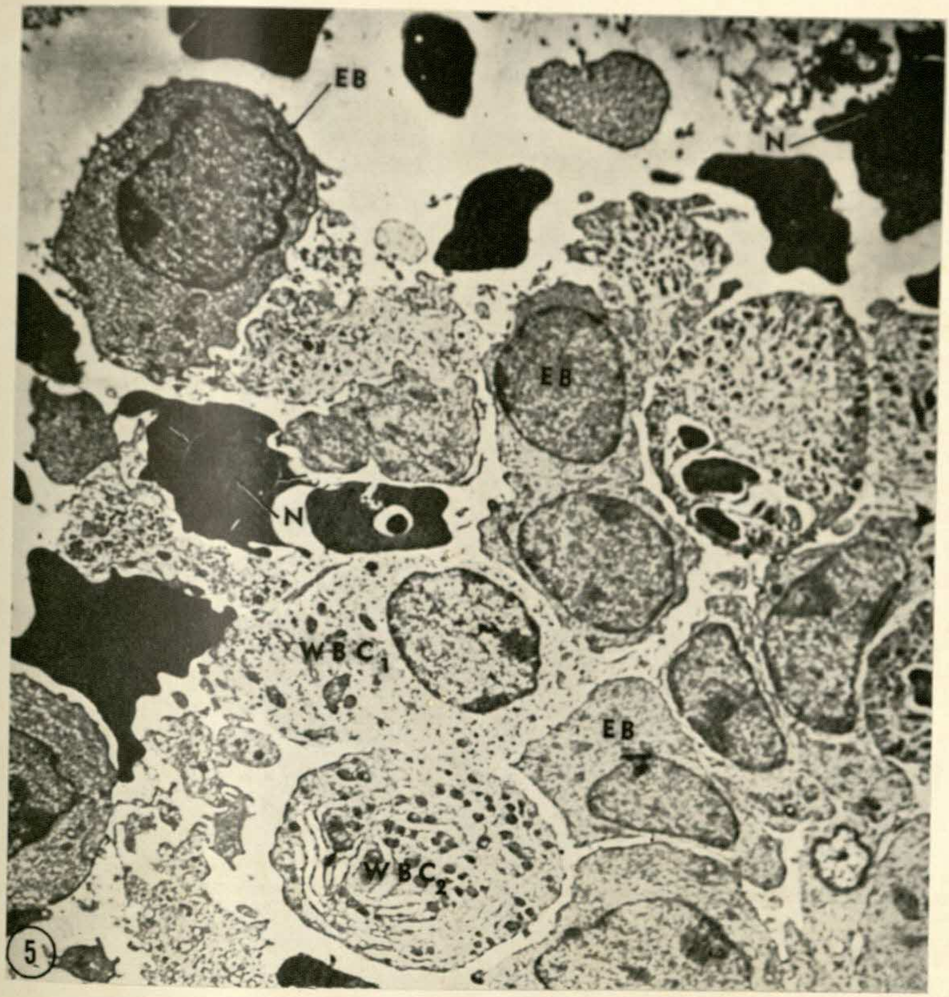
The density of the erythroblastic cells (EB) distinguishes them from the other marrow cells (WBC 1 and 2), many of which have cytoplasmic granules and complex endoplasmic reticulum (WBC 2), but some are undifferentiated (WBC 1). The late normoblasts (N) are characterized by extreme electron density which matches that of the mature erythrocyte. The three labelled early normoblasts (EB) show an increase in their density which affects both nucleus and cytoplasm. In the late normoblast (N upper right) it is possible to distinguish nucleus and cytoplasm, and an artificial cleft at the nucleo-cytoplasmic boundary has made this distinction more obvious. A similar artefact in the late normoblast (N centre left) has revealed an otherwise indistinguishable nucleus.

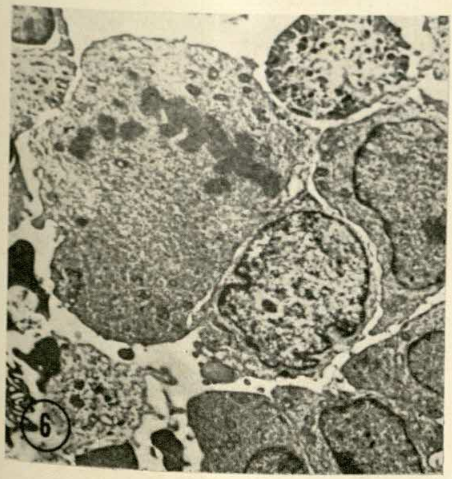
Fig. 6.—As fig. 5.

An early normoblast is in mitosis. The nuclear membrane is absent and the chromosomes are surrounded by cytoplasm containing hæmoglobin.

Fig. 7.—Adult human circulating blood, from a case of myelofibrosis with many normoblasts in the peripheral blood. Dalton fixation. × 8000.

A late normoblast is shown in which a very indistinct nuclear membrane (arrows) outlines the nucleoplasm which is similar to the surrounding cytoplasm.


EB
N
EB
N
WBC$_1$
EB
WBC$_2$
5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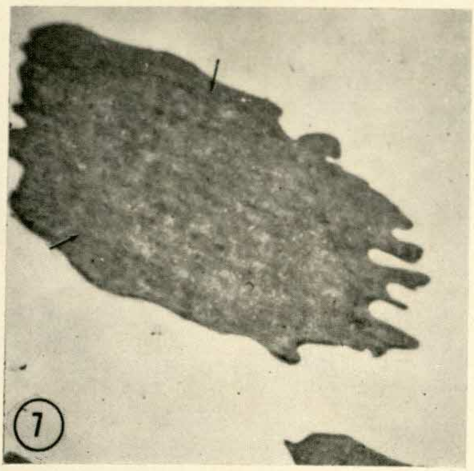
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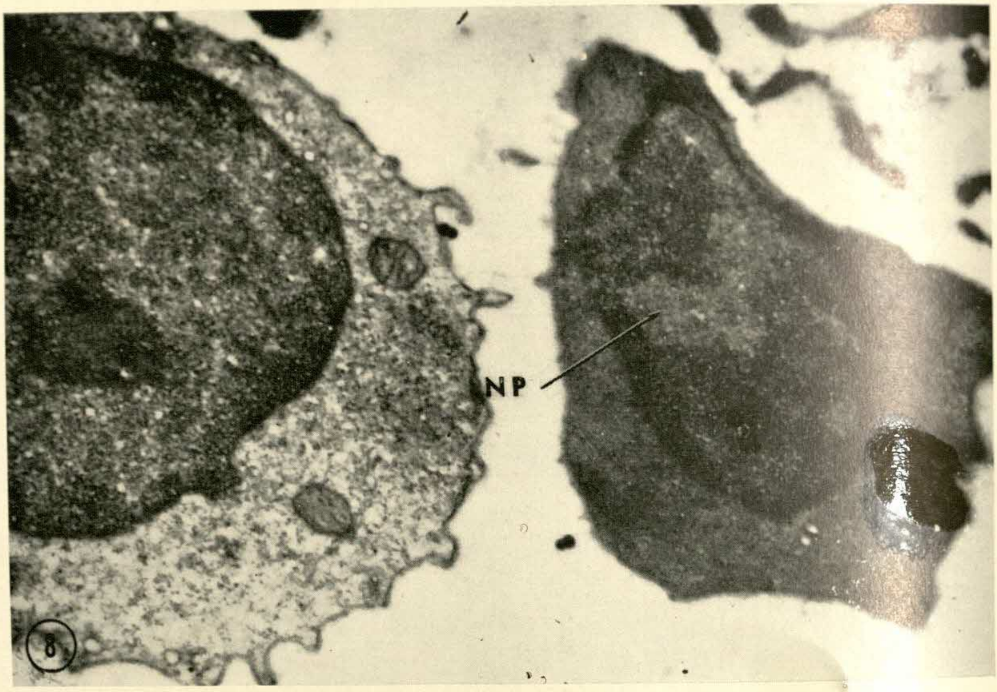
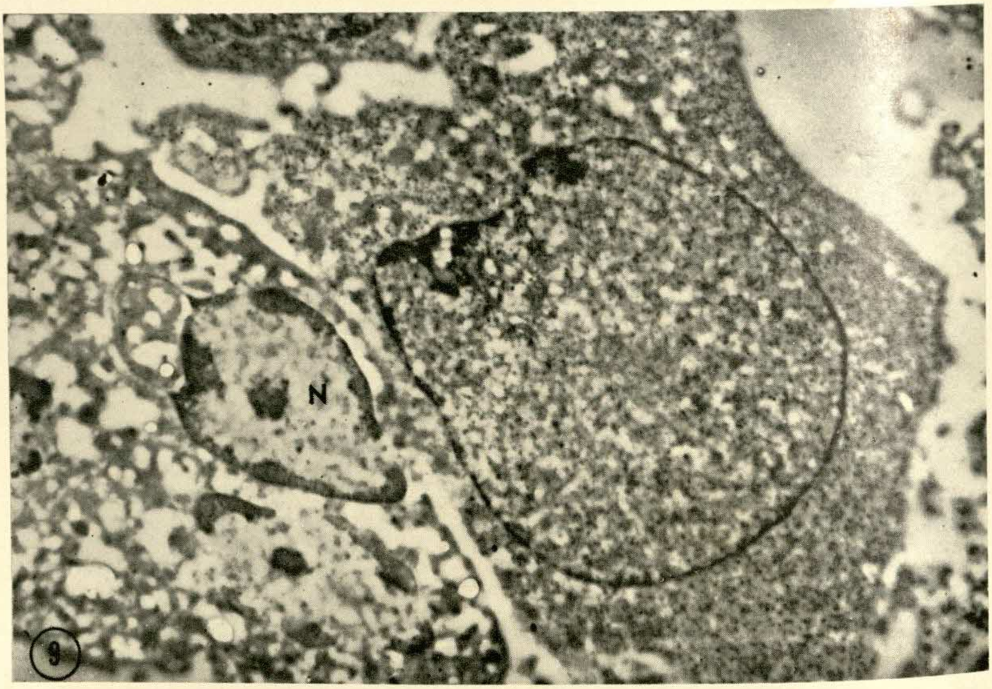

NP
8


N
9

In contrast to the smooth outline of the early erythroblast, whether embryonic or adult, the late normoblast frequently has a cell membrane arranged in irregular folds (fig. 7). This observation is consistent with the motility of the normoblast which has been observed in phase contrast studies of the living cell [Bessis and Bricka, 1952].

In electron micrographs of bone marrow occasional normoblasts in mitosis are seen. In such cells, without a nuclear membrane, the chromosomes can be recognized lying in a mass of protoplasm which has a uniform structure throughout (fig. 6).

In electron microscopy many fewer cells are examined than in light microscopy and negative observations are of limited value. Little significance can therefore be attached to the paucity of naked nuclei in our specimens, but it is of interest that the only one we observed does not have the density which we associate with the late normoblast nucleus.

DISCUSSION

On the basis of electron absorption it is suggested that, during maturation of the erythroblast, hæmoglobin or associated iron containing compounds are deposited in the nucleus as well as in the cytoplasm. This possibility is considered by a number of workers using several methods of investigation.

Using histochemical methods, Garcia-Blanco and Forteza-Bover [1946] demonstrate what is believed to be hæmoglobin in the parachromatin of the more immature erythroblasts, though not in the late normoblast.

Because of its characteristic absorption spectrum, intracellular hæmoglobin can be estimated quantitatively by microspectroscopy. The estimation of nuclear content by this method is complicated by the light which traverses the nucleus having to pass through two layers of cytoplasm as well. In living cell suspensions it can be assumed that the erythroblasts and their nuclei are spherical and correction can be made for the absorption due to the cytoplasm. Using this method Thorell [1947, 1955] reports rather equivocal results; his latest opinion [1955] is that up to ten per cent of the hæmoglobin of the normoblast is contained in the nucleus. In cell smears there is only a thin layer of cytoplasm above and below the nucleus; using such preparations, Metcalf [1951] and Wilkins and de Carvalho [1953] show absorption due to hæmoglobin in the nuclear area of smeared erythroblasts.

Stern *et al.* [1951] report that hæmoglobin makes up nineteen per cent of the dry weight of isolated nuclei from fowl red cells.

FIG. 8.—Adult human circulating blood, from a case of acute leukæmia. Dalton fixation. ×12,000.

A lymphocyte is seen on the left and a late normoblast on the right. There are aggregations of denser material in the normoblast nucleus, but the intervening nucleoplasm (NP) is similar to the neighbouring cytoplasm.

FIG. 9.—Adult human bone marrow, normoblastic erythropoiesis. Fixed in 10 per cent formalin in normal saline for 1 hr. ×10,000.

A normoblast is seen on the right and a polymorphonuclear leucocyte on the left. A linear nuclear membrane separates structurally identical nucleoplasm and cytoplasm; these are denser than the corresponding parts of the neighbouring white cell.

Schmid, Schwartz and Sundberg [1955] report a careful study of the site of hæmoglobin formation in congenital porphyria—a rare abnormality of porphyrin metabolism. Using histochemical staining, microspectroscopy and fluorescence microscopy they demonstrate the presence of hæmoglobin and abundant porphyrins in the nuclei of erythroblasts in this condition, though no observations on normal controls are reported.

The shape of the cell and the limited resolving power make it impossible, at the moment, to demonstrate the intracellular site of ^{59}Fe uptake in autoradiographs. Lajtha [1957] using autoradiographs of marrow blood smears, after culture in the presence of ^{59}Fe, reports that naked nuclei do not show any radioactivity. He suggests that this may be due to the leaching of hæmoglobin, containing ^{59}Fe, from the exposed nuclei.

As hæmoglobin is deposited in the nucleus the difference between the nucleus and the cytoplasm, as seen in the electron micrograph, diminishes and the nucleo-cytoplasmic boundary disappears. This observation supports the view that the nucleus is not extruded as a whole from the normoblast, but the possibility that parts of the nuclear material are extruded cannot be excluded on the basis of static electron microscopic observations. Pease [1956] shows electron micrographs of guinea-pig bone marrow illustrating normoblasts with extremely eccentric nuclei, which he considers to be evidence for the extrusion of the entire nucleus. But the normoblasts which Pease illustrates as extruding their nuclei are much less mature than the late normoblasts in which we demonstrate the continued presence of a nucleus.

Cajal [1933] reports his earlier observations of nuclear extrusion from normoblasts, *in vitro*, and recently Bessis and Bricka [1952] have confirmed this phenomenon using a warm stage phase microscope. But the fate of the normoblast nucleus has been a matter of controversy for over half a century and light microscopists remain divided on this issue [Dickson, 1908; Isaacs, 1938; Albrecht, 1951; Whitby and Britton, 1953]. Our contribution to this controversy is the demonstration of hæmoglobin deposition in the nucleus and the disappearance of the nucleo-cytoplasmic boundary.

A parallel to the maturation of the normoblast is to be found in the development of the stratum corneum in a stratified squamous epithelium; where the nucleus disappears and there is no suggestion of nuclear extrusion during this process. In an electron microscopical study of oral epithelium Sognnaes and Albright [1956] show the presence of kerato-hyaline granules not only in the cytoplasm but also within the nucleus of cells in the stratum granulosum. This observation has been confirmed by one of the authors (A. R. M.) in the epithelium of the rat's œsophagus.

Caspersson [1950] was the first to suggest that the nucleolus plays an important part in ribonucleic acid synthesis. Large nucleoli are seen in the early erythroblast at the stage when the cytoplasm contains the small dense granules which are considered by Palade [1955] to be mainly ribonucleic acid and to be responsible for basophilia. It is well known that the characteristic basophilia of the early erythroblast disappears as hæmoglobin appears, and Thorell [1947] has shown by ultra-violet light absorption that cytoplasmic ribonucleic acid diminishes before hæmoglobin can be detected in the cell. This author suggests that the globin is formed during the growth of the cell at the stage when cytoplasmic ribonucleic acid is plentiful and that the hæm is conjugated later, after the disappearance of the ribonucleic acid. This explanation is supported by our observation that neither endoplasmic reticulum nor ribonucleic acid granules are present in cells containing more than a trace of hæmoglobin.

Although there is much evidence [Caspersson, 1950; Palade and Siekewitz, 1956; Weiss, 1953] to suggest that protein synthesis occurs in the presence of ribonucleic acid, our observations, together with those of Thorell suggest that it is not necessary for hæm synthesis. Our observations do not indicate the intracellular site of hæmoglobin synthesis, but we note that the concentration in the cytoplasm is greater than that in the nucleoplasm in embryonic cells.

It is possible that some hæmoglobin enters the nucleus while the nuclear membrane is absent during mitosis, but the increase in concentration of hæmoglobin in the nucleus continues beyond the stage of maturation at which mitosis is normally seen. Part of this late increase in density may be explained by shrinkage of the cell and nucleus but it appears to be too great to be explained entirely on this basis.

ACKNOWLEDGMENTS

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STUDIES ON THE MECHANISMS IN THE DUODENUM INHIBITING GASTRIC SECRETION.

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Dogs were provided with denervated, innervated and autotransplanted gastric pouches. Cannulation of duodenum and jejunum, explanation of a section of duodenum, cannulation of an isolated duodenal loop, and excision of the antrum were variously associated in different groups of the pouch-equipped dogs.

The secretion of acid from the pouches was stimulated by meat or histamine and the effects on this secretion of various substances applied to the duodenum and jejunum were investigated. Many new facts have emerged.

Acids, hypertonic saline, sugars, peptone and fat can inhibit the gastric response to histamine. Two separate inhibiting mechanisms operate from the duodenum, one receptive to changes in pH the other to changes in osmolarity. The initiation of inhibition depends on a critical level of either pH or osmolarity. The pH sensitive mechanism exerts its effect through neural pathways and the osmolar sensitive mechanism through a humoral pathway. The antrum of the stomach is not a necessary part of these inhibition processes. The receptors for both mechanisms appear to be confined to the duodenum. Whereas sugars and peptone act directly in promoting inhibition, prior digestion of fats appears to be necessary.

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SINCE the demonstration over a half-century ago that the placing of hydrochloric acid [Shemiakin, 1901] or of gastric juice [Sokolov, 1904] in the duodenum of dogs inhibited meat-stimulated secretion from innervated gastric pouches, there have been a number of studies confirming the presence in the duodenum of mechanisms capable of inhibiting gastric secretion.

In dogs the mechanism is excited by the presence of fat [Pavlov, 1910; Kosaka and Lim, 1930; Feng, Hou and Lim, 1929; Gray, Bradley and Ivy, 1937], of hydrochloric acid [Sokolov, 1904; Crider and Thomas, 1932; Day and Webster, 1935; Wilhelmj, McCarthy and Hill, 1937; Pincus, Earl Thomas, Friedman and Rehfuss, 1944], and of carbohydrates [Day and Komarov, 1939]. All three phases of the gastric secretory response to meals are affected but it was reported that the secretory response to histamine was not influenced [Day and Komarov, 1939; Pincus *et al.*, 1944]. However, since the present study was commenced, Code and Watkinson [1955] demonstrated that the response of innervated gastric pouches to histamine could be inhibited by acid in the duodenum.

In the duodenum of man a similar mechanism has been shown to exist and to be excited by acid [Griffiths, 1936; Shay, Gershon-Cohen and Fels, 1942] and by glucose and fat [Shay *et al.*, 1939].

But of neither species does there exist any detailed knowledge of how the mechanisms operate, or, indeed, if more than one mechanism is involved. It is known that acetic or sulphuric acid can be as equally effective as hydrochloric [Vandorfy, 1924 and 1925; Farrell, 1928; Goldberg, 1932] and that the pH must be below 2·5 [Pincus *et al.*, 1944]. According to Code and Watkinson [1955] acid in the duodenum will only inhibit the secretion from gastric pouches which have an intact supply from the vagus nerve. It has also been claimed that the acid-excited mechanism does not operate if acid regurgitation to the antrum is prevented [Woodward, *et al.*, 1954]. Under certain conditions, acid in the small intestine has been shown to *stimulate* the secretion of acid from gastric pouches [Sircus, 1954; Code and Watkinson, 1955].

Nothing is known of the effect upon gastric secretion of protein material in the duodenum, but semi-digested meat chyme placed in the jejunum stimulates the secretion of acid from gastric pouches [Sircus, 1954].

This study was undertaken in an attempt to discover what mechanisms capable of inhibiting gastric secretion exist in the duodenum of dogs, how they work, and if they are confined to that part of the small intestine.

METHODS

1. *Operative Procedures* (fig. 1).—Dogs were provided with either innervated, denervated or autotransplanted pouches of the fundus of the stomach. In all the dogs a cannula was placed in the duodenum, and in some dogs another in the jejunum.

In three of the dogs the antrum of the stomach was subsequently resected. Two other dogs, in place of cannulation, had an explantation of the duodenum to the abdominal wall. In one dog with an innervated pouch, a Thiry-Vella loop 12 cm. in length was fashioned entirely from the duodenum and cannulated at both ends.

The cannulæ were made from silver and those in the intestine were provided with screw caps. The duodenal cannulæ, other than those in the Thiry loop, were placed within 3 cm. of the pylorus and those in the jejunum between 5 and 10 cm. distal to the ligament of Treitz.

The autotransplanted pouches were made by the method of intramammary implantation [Gregory and Ivy, 1941]. The duodenal explants were prepared by transection of the duodenum immediately below the common bile duct and accessory pancreatic duct, and again 10–12 cm. distally. The main pancreatic duct was ligated and the blood supply to the isolated duodenal segment was carefully preserved. After restoring the continuity of the duodenum by end-to-end anastomosis, the excluded segment was brought outside the abdomen, cut along the antimesenteric border, opened out and sewn into the abdominal wall in place of a previously excised rectangle of skin of similar area.

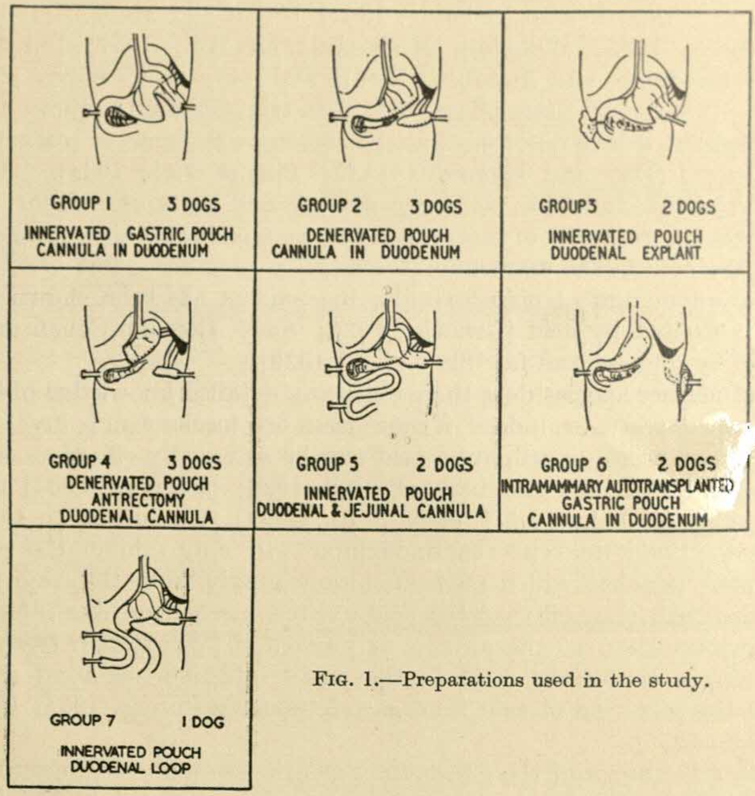


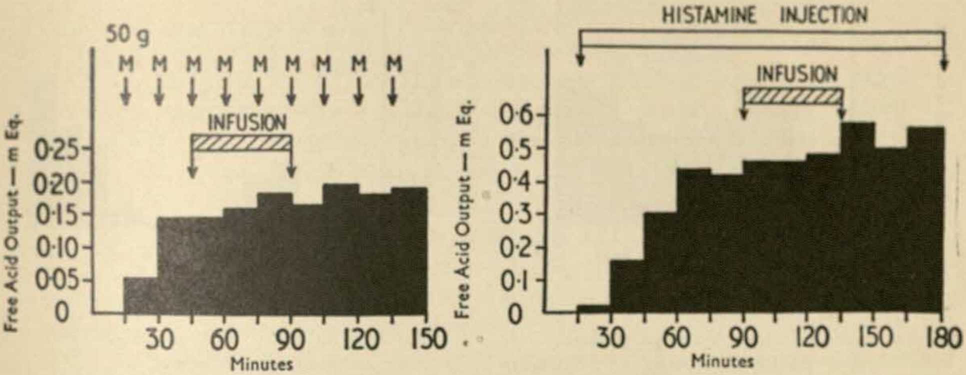
FIG. 1.—Preparations used in the study.

2. *Experimental Procedure.*—Gastric secretion was stimulated by feeding either 25 or 50 g. of a standard meat preparation at intervals of 15 min., or by continuous subcutaneous injection of histamine acid phosphate using a motor-driven ram piston injector with a variable speed. The amount of histamine injected was that calculated to produce a response approximately half of the maximum possible output from the pouch being stimulated, and was of the order of 1·0–3·0 mg. of histamine acid phosphate per hour of delivery. Infusions into the duodenum were made from a heated reservoir with thermostat and rate of flow controls, and connected by polythene tubing to the intestinal cannulæ. Delivery of infusates was at 37° C.,

and the rate varied from 15 to 100 ml. in 15 min. in different experiments. Gastric secretion was collected in graduated tubes in 15-min. fractions, and titrated against N/10 caustic soda using Topfer's reagent as indicator.

Because of the known effect of nausea on gastric secretion [Grossman, Woolley, Dutton and Ivy, 1945] experiments were abandoned if salivation, lip-licking or retching occurred.

The infusates used in the study were tap-water, normal and hypertonic saline, hydrochloric acid of pH ranging from 1·5 to 2·5; glucose in 5, 10, 25 and 40 per cent solutions; fructose in 2·5, 5 and 10 per cent solutions; dextran, 6 and 10 per cent solutions; peptone, 2·5, 5, 10 and 20 per cent solutions; 25 and 40 per cent emulsions of olive oil; and digests of olive oil with pancreatic juice.



Pouch innervated

Pouch denervated

Duodenal infusions: normal saline 100 ml./15 min.

Fig. 2.—The normal responses of gastric pouches to meat meals and to continuous subcutaneous injection of histamine. Control infusions of normal saline into the duodenum fail to inhibit the responses.

Before each experiment the dogs fasted for at least 12 hr. and secretion from the pouch was minimal in volume and free acid was always absent. The secretion was measured for a control period of at least 15 min. before commencing stimulation. Infusions into the duodenum were begun when the secretory response to the histamine or meat had become steady.

In a number of experiments with duodenal explants 5 per cent procaine solution was applied directly to the mucosa. This method has been shown to block the excitation of local neural mechanisms involved in the stimulation of secretion and motility in the stomach and small intestine [Zeliony and Savich, 1911; Lim, Ivy and McCarthy, 1925; Gregory and Ivy, 1941; Sircus, 1954].

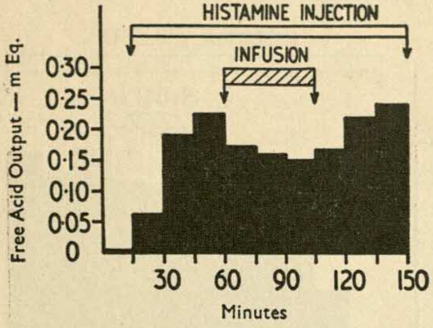
Results

1. *The Inhibition of Gastric Secretion by Intraduodenal Infusions*

(a) *Control Tests with Normal Saline.*—Twenty-five experiments were made on 10 dogs with either innervated or denervated gastric pouches. The stimulus to secretion was provided by meat in 5 experiments and by histamine in the remainder. From 50 to 100 ml. of normal saline were infused into the duodenum every 15 min. for up to 45 min. (fig. 2*a* and *b*).

Inhibition of the secretion from the pouch was observed on only one occasion. In a few instances, and usually where meat provided the stimulus, a recognizable, but slight, augmentation of the secretory response occurred.

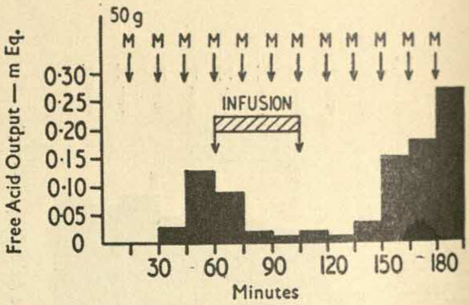
(b) *The Infusion of Hypertonic Saline.*—Eighteen experiments were made on 8 dogs with innervated or denervated gastric pouches. In each, histamine was used as stimulus. Inhibition of the acid secretory response was observed in 11 experiments in which saline, 5 per cent W/V, was infused. No effect was observed in the other 7 instances, and no difference of effect appeared between the two types of gastric pouch (fig. 3).



Pouch denervated

Duodenal infusion: hypertonic saline 20 ml./15 min.

FIG. 3.—The effect of hypertonic solution in the duodenum upon the gastric response to histamine.



Pouch innervated

Duodenal infusion: 40 per cent glucose 50 ml./15 min.

FIG. 4.—The effect of duodenal infusion of hypertonic solutions of glucose upon the gastric response to meals.

Inhibition appeared within 15 min. of commencing the infusion and persisted for more than 15 min. after it was stopped.

(c) *The Infusion of Carbohydrates.*—

(i) *Glucose:* 32 experiments were made on 10 dogs with innervated or denervated pouches.

Meat was used as stimulus in 4 experiments, and histamine in the others.

Glucose in concentrations of 5, 10, 25 and 40 g. in 100 ml. of normal saline was infused into the duodenum.

Inhibition of the pouch response was observed in 25 of the experiments and no effect in the remainder. It appeared within 15 min. of commencing the duodenal infusion and persisted for 30 min. or more after it stopped. The response to meat and to histamine were both inhibited.

The degree of inhibition appeared, in general, to be related to the total amount of solute infused, and not to its concentration in the infusate. While the most marked inhibition appeared when 40 per cent glucose was infused at the rate of 50 ml. in 15 min. (20 g. in 15 min.) (fig. 4), the same concentration of glucose excited no effect on gastric secretion when it was infused at the rate of 10 ml. in

15 min. (4g.). However, 5 per cent glucose solution did inhibit gastric secretion on one occasion when infused at 50 ml. in 15 min. (2·5 g.). The extent of inhibition varied considerably. Determining the degree of inhibition by a comparison of the output of free acid from the pouch 1 hr. before commencing the infusion with that in the subsequent hour, 50 g. of glucose infused over 45 min. inhibited the response to meat by 86 per cent, and to histamine by 54 per cent (fig. 5).

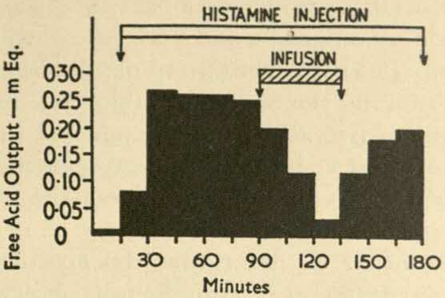
In order to determine the specificity of the action of carbohydrates in the duodenum, observations were made on the effects of infusion of another monosaccharide, fructose, and of a polysaccharide complex, dextran.

(ii) *Fructose:* 7 experiments were made on the response to histamine in 4 dogs. Inhibition appeared in 5 when 5 or 10 per cent solutions were infused (fig. 6), but did not appear on the two occasions when 2·5 per cent solution was infused at a rate of delivery of 12·5 ml. in 15 min. (0·3 g.). The *minimum* amount of solute delivered in 15 min. which resulted in inhibition was 1·2 g. Under the conditions of that experiment the degree of inhibition, calculated as stated above, was 39 per cent.

(iii) *Dextran:* 4 experiments were made on 2 dogs with denervated pouches stimulated by histamine. Inhibition was obtained on each occasion that dextran was infused but it was not marked and was transient. Thus the infusion of 14·4 g. over 45 min. (6 per cent solution at 6 ml./min.) produced an inhibition of 24 per cent which was not prolonged beyond the period of infusion (fig. 6). Two experiments were made on a dog with an innervated pouch stimulated by meat. In both cases inhibition by 10 per cent dextran was pronounced, amounting to 50 per cent and persisting for an hour after perfusion. The osmolar values of the solutions used were: 290 mOsm./l. and 480 mOsm./l. for the 6 and 10 per cent solutions respectively.

(d) *The Infusion of Fats.*—Twelve experiments were made on 5 dogs with duodenal cannulation and innervated or denervated pouches, and normal alimentary continuity. Olive oil in 25 or 40 per cent emulsion was infused in 9 instances, and a 50 per cent emulsion of ground-nut oil ("prosparol") in the other 3. Meat provided the stimulus in 4 experiments and histamine in the remainder.

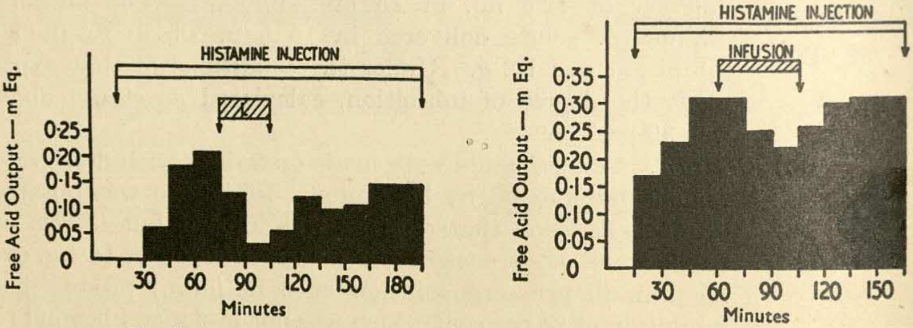
Marked inhibition appeared in 11 of 12 experiments and no effect in the other, in which ground-nut oil had been used. The inhibition by fat of the response to meat was the most marked of the whole study and in some instances was virtually absolute for long periods. With both meat and histamine stimuli it appeared after an interval of 10 min. and persisted for up to 90 min. after stopping the duodenal infusion. No difference was



Pouch denervated

Duodenal infusion: 40 per cent glucose
50 gm. in 45 min.

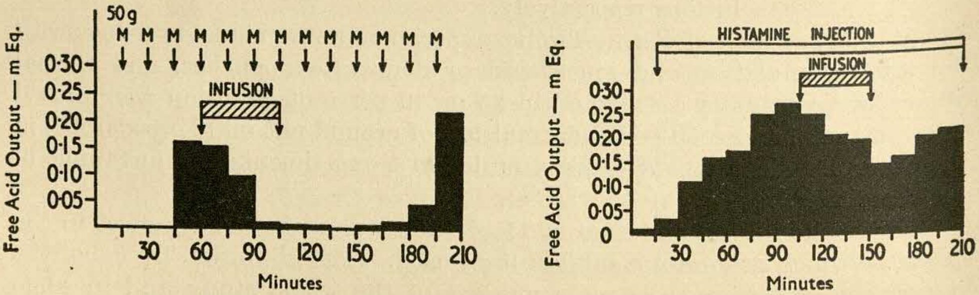
FIG. 5.—The effect of duodenal infusion of hypertonic solutions of glucose upon the gastric response to histamine.



Pouch denervated
Duodenal infusion: 5 per cent fructose
50 ml./30 min.

Pouch denervated
Duodenal infusion: 6 per cent dextran
90 ml./45 min.

Fig. 6.—The effect on the gastric response to histamine of duodenal infusions of fructose and dextran. Other monosaccharides and polysaccharide complexes are as effective as glucose in inhibiting gastric secretion.



Pouch innervated

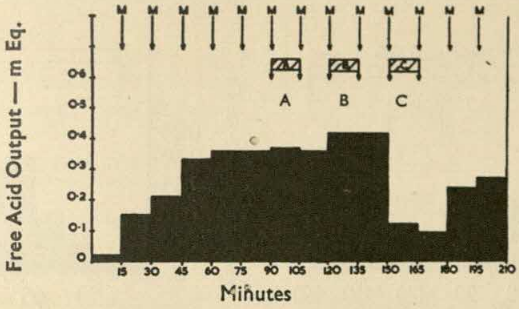
Pouch denervated

Duodenal infusions: 40 per cent emulsion of olive oil

FIG. 7.—The effect of duodenal infusion of olive oil on the gastric response to meals and to histamine.

observed between the effects in denervated and innervated pouches (fig. 7). As little as 10 ml. of 50 per cênt emulsion of ground-nut oil infused over 15 min. was sufficient to induce inhibition, but it was most pronounced when 45 ml. of 40 per cent emulsion of olive oil were infused in 15 min. However, when similar fat emulsions were perfused through the isolated duodenal loop preparation inhibition of the secretory response of the innervated pouch in that dog failed to appear in any of 4 experiments.

Three experiments were then made on the same preparation in which olive oil was perfused after it had been incubated for 2 hr. at 37° C. in a mixer with pancreatic secretion obtained from a dog with a pancreatic fistula. In all 3 experiments clear evidence of inhibition of the gastric secretory response to meals was obtained (fig. 8). Prior control perfusions of incubated olive oil alone, and incubated pancreatic juice alone, failed to produce any evidence of inhibition of gastric secretion.



Pouch innervated
Isolated duodenal loop

Duodenal infusions:
Soln. A: 15 ml. pure oil after 2 hr. at 37° C.
B: 15 ml. pancreatic juice after 2 hr. at 37° C.
C: 10 ml. olive oil + 5 ml. pancreatic juice after 2 hr. at 37° C. in mixer.

FIG. 8.—The acid inhibiting action of fats appears to be dependent on a digestion product.

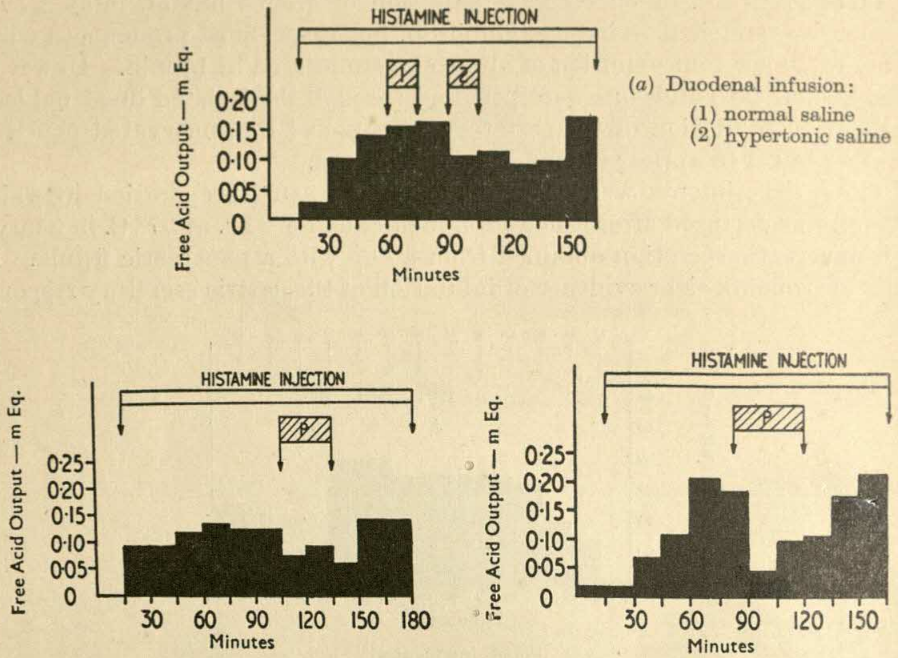
The perfusion of glycerol (2 experiments), oleic acid (2 experiments), and linoleic acid (2 experiments) through the same preparation has resulted on every occasion in a slight *augmentation* of gastric secretion from the innervated pouch.

(e) *The Infusion of Protein.*—Thirteen experiments were made on 4 dogs infusing 2·5, 5 and 10 per cent solutions of "oxoid" brand bacteriological peptone, with histamine as the stimulus to gastric secretion.

Inhibition appeared in 8 instances, and augmentation of secretion in 2 others. No effect was observed on 3 occasions.

It became clear that the higher the concentration of peptone, the greater the degree of inhibition of gastric secretion, and this graduated effect is demonstrated by the results on gastric secretion of delivering 0·65 and 1·25 g. of peptone in 15-min. periods to the duodenum of the same dog (fig. 9*a*, *b*, *c*).

Pouch denervated: same dog all 3 experiments.

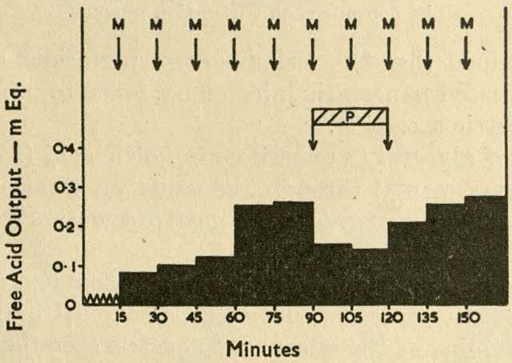


(*a*) Duodenal infusion:
(1) normal saline
(2) hypertonic saline

(*b*) Duodenal infusion: 2·5 per cent peptone

(*c*) Duodenal infusion: 5 per cent peptone

Fig. 9.—The degree of inhibition of gastric secretion by solutes in the duodenum is related to the osmolarity of the infusate.

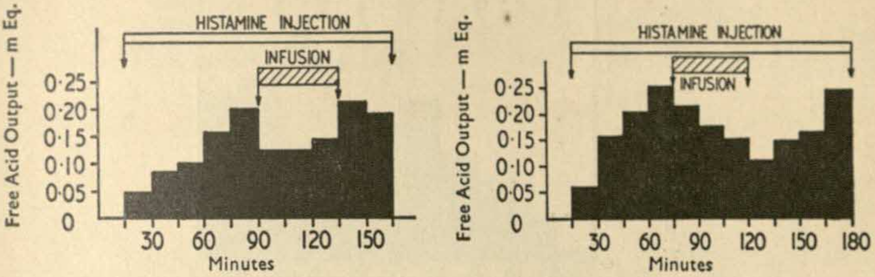


Pouch innervated
Isolated duodenal loop
Duodenal perfusion: 30 per cent peptone

Fig. 10.—The effect upon the gastric response to meals of the perfusion of the isolated duodenum with peptone.

Inhibition of gastric secretion was also observed when the isolated duodenal loop preparation was perfused with peptone solutions (fig. 10).

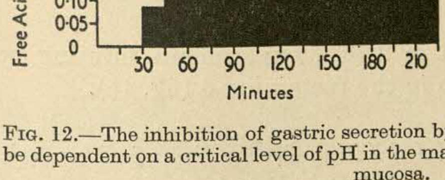
(f) *The Infusion of Hydrochloric Acid.*—Thirty-one experiments were made on 9 dogs with innervated or denervated pouches, and acid of pH ranging from 2·5 to 1·5 at a rate of delivery varying from 10 to 90 ml. per 15 min. was infused into the duodenum. A sharp difference was observed between denervated and innervated pouches in the effects upon gastric secretion.



Pouch denervated
Duodenal infusion: HCl pH 1·5
60 ml./15 min.
This order of inhibition in one-third of experiments on denervated pouches.

Pouch innervated
Duodenal infusion: HCl pH 1·5
60 ml./15 min.

FIG. 11.—The effect of duodenal infusion of acid on the response to histamine of denervated and innervated gastric pouches.



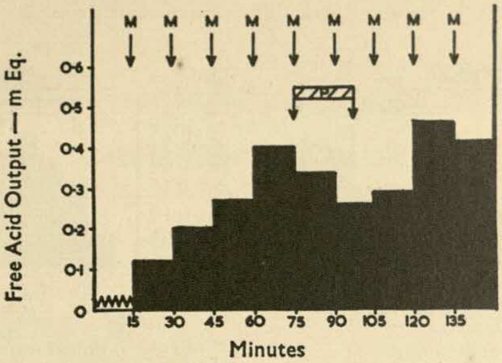
Pouch innervated
Duodenal explant
Continuous drip of solutions on to exposed duodenal mucosa.
pH recorded in overflow from mucosa.
S = normal saline
A1 = overflow at pH 2·0
A2 = overflow at pH 1·8

FIG. 12.—The inhibition of gastric secretion by acid in the duodenum appears to be dependent on a critical level of pH in the material in contact with the duodenal mucosa.

Of 18 experiments on dogs with denervated pouches inhibition was observed in only 7 instances and was slight and transient (fig. 11). In 16 experiments on dogs with innervated pouches inhibition appeared in 14 and was marked and prolonged (fig. 11).

The responses to both meat and histamine were affected by the infusions and inhibition appeared to be dependent on a critical level of pH at the surface of the duodenal mucosa (fig. 12).

The perfusion of an isolated duodenal loop with hydrochloric acid on 3 occasions each time resulted in clear inhibition of the response of the innervated pouch in the same dog (fig. 13).



Pouch innervated
Isolated duodenal loop
Duodenal perfusion (P) = HCl pH 1·5

FIG. 13.—The effect of perfusion of an isolated duodenal loop with hydrochloric acid upon the response to meals of an innervated pouch.

2. *The Effect of Removal of the Gastric Antrum on the Inhibiting Mechanisms*

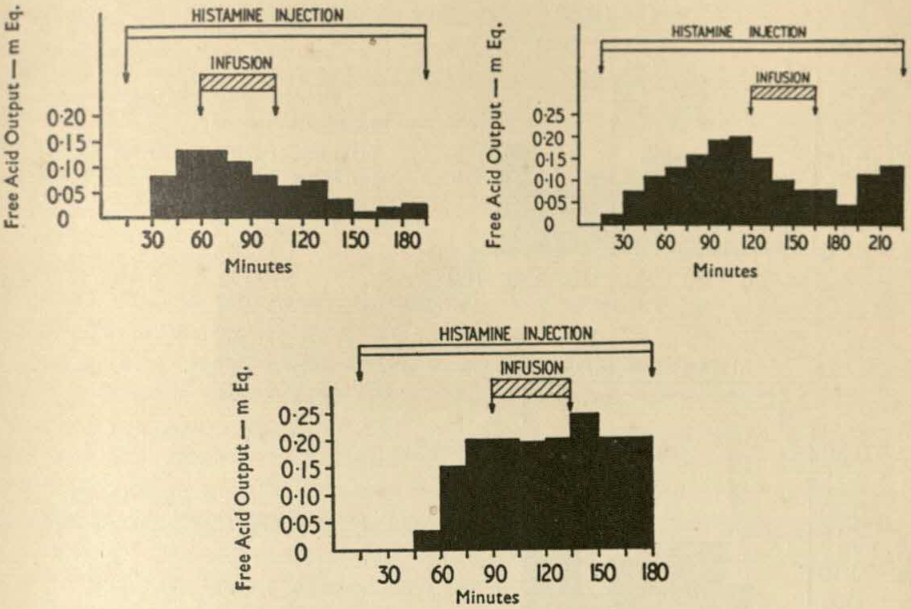
Excision of the gastric antrum with end-to-end anastomosis of the duodenum to the body of the stomach was carried out on 3 dogs with denervated pouches and cannulated duodenum. The effects of duodenal infusions of normal and hypertonic saline, glucose, fructose, fats, peptone and hydrochloric acid were again observed. In all of 6 experiments normal saline had no effect on the response to histamine, hypertonic saline caused inhibition in 3 of 5 experiments, glucose and fructose in 7 of 13 experiments, and olive oil in both of 2 experiments (fig. 14*a*, *b*).

No inhibition of any degree was obtained in 6 experiments infusing hydrochloric acid (fig. 14*c*).

Slight inhibition was observed in 2 of 7 experiments infusing peptone, and clear *augmentation* of secretion in the remaining 5 (fig. 15).

3. *The Operation of Duodenal Inhibiting Mechanisms in Dogs with Autotransplanted Gastric Pouches*

As the autotransplanted gastric pouch is completely separated from its original blood and nerve supply, any influence on it, effected from a distance, can only be exerted through the medium of either a humoral agent, or of alteration in the dynamics of the blood flow to the peripheral tissues. For this reason the autotransplanted pouch has been accepted as a reliable tool for the investigation of hormonal factors in gastro-intestinal physiology [Gregory and Ivy, 1941].



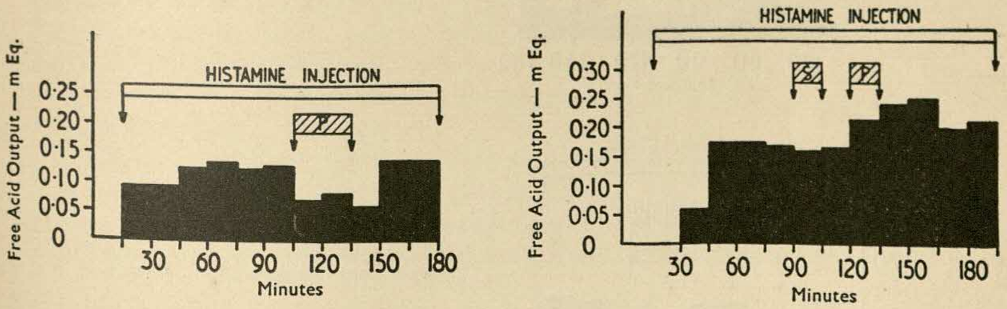
Pouch denervated
Antrectomy

(*a*) Duodenal infusions: hypertonic saline 25 ml./15 min.

(*b*) Duodenal infusion: 40 per cent glucose 40 ml./15 min.

(*c*) Duodenal infusion: HCl pH 1·5
No inhibition in all experiments

FIG. 14.—The inhibition of gastric secretion by saline, glucose and fat solutions in the duodenum is unaffected by antrectomy and pouch denervation, but the action of acid is thereby eliminated.

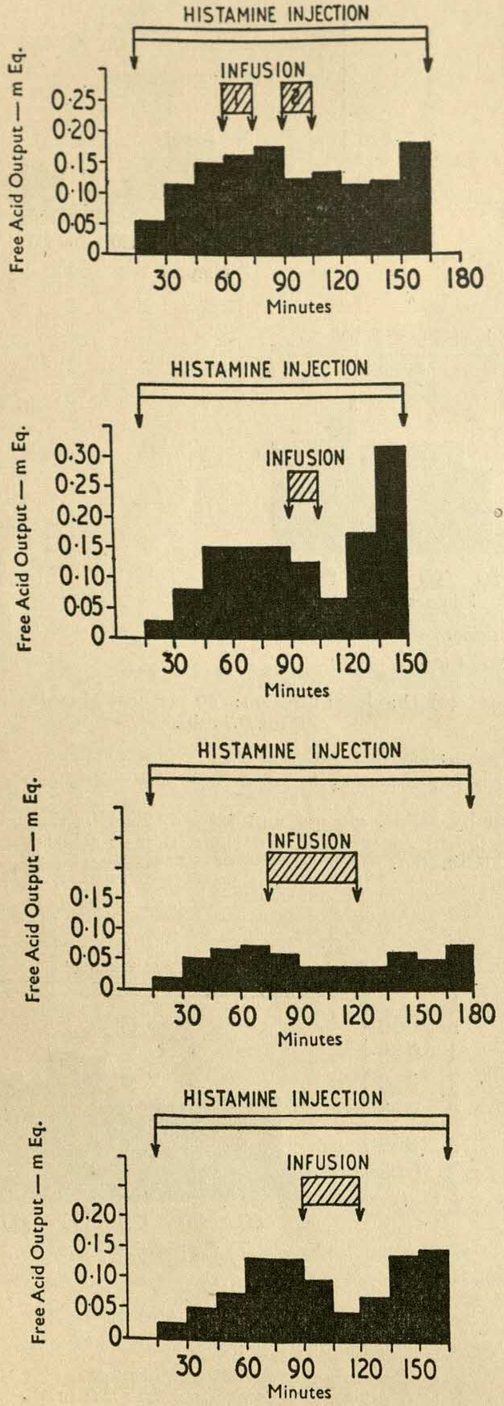


Pouch denervated

(*a*) With antrum intact
Duodenal infusion (P): 2·5 per cent peptone 25 ml./15 min.

(*b*) After removal of antrum
Duodenal infusions:
S = normal saline 25 ml./15 min.
P = 2·5 per cent peptone 25 ml./15 min.

FIG. 15.—Following removal of the antrum peptone solutions infused into the duodenum appear to augment the response of gastric pouches to histamine.



Pouches autotransplanted
Duodenal infusions:
(1) normal saline 50 ml./15 min.
(2) hypertonic saline 50 ml./15 min.

10 per cent peptone 30 ml./15 min.

40 per cent glucose 20 ml./15 min.

10 per cent lævulose 25 ml./15 min.

Fig. 16.—The inhibition of gastric secretion by hypertonic solutions in the duodenum appears to be dependent on a humoral mechanism as it is effective in dogs with intramammary autotransplanted pouches.

Twenty-two experiments were made on 2 dogs using histamine as the stimulus (fig. 16).

Three out of 4 control infusions of normal saline into the duodenum had no inhibiting effect on the pouch responses, 1 causing slight augmentation of secretion.

Of 5 infusions of 5 per cent saline, inhibition appeared in 3, and 2 had no effect.

Glucose, infused in 40 per cent solution, caused inhibition in 4 of 6 experiments, and fructose 10 per cent, in all 3 in which it was used.

Peptone 10 per cent was twice infused and caused recognizable inhibition on both occasions (fig. 11).

The secretory responses from autotransplanted pouches is much less than that from simple denervated (Heidenhain) pouches, and it was noticeable that when inhibition did appear, it was never marked and often short lived. Thus in 1 experiment the infusion of 10 per cent peptone (3 g. in 15 min.) caused an inhibition of 36 per cent in the 30 min. subsequent to commencing the infusion compared with the previous 30 min., but was succeeded thereafter by 30 min. of rebound secretion in which the output of hydrochloric acid was 70 per cent above that of the control period (fig. 11).

The infusion of 0·15 N hydrochloric acid on 3 occasions had no effect on the response of the pouch to histamine.

4. *The Effect of Prior Anæsthetization of the Duodenal Mucosa on the Responses to Glucose and Acid*

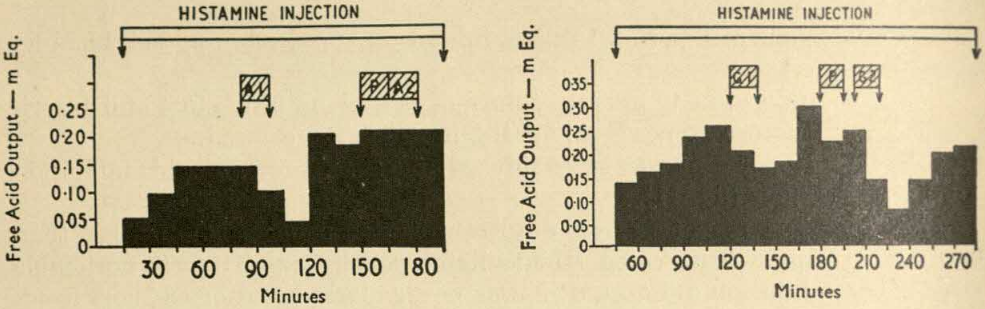
Eleven experiments were made on 2 dogs with a segment of duodenum implanted into the abdominal wall and with innervated gastric pouches. After the response of the pouch to histamine had become steady a 5 per cent solution of procaine hydrochloride (B.P.) was applied directly to the mucosa by means of a gauze swab shaped to the implant, soaked in the solution, and kept moist by a continuous drip of procaine. After 15 min. this was removed and either hydrochloric acid or glucose solution was allowed to flow continuously over the duodenal mucosa. The pH of the solution in direct contact with the mucosa was estimated by measuring that of the overflow from the implant.

In all 6 experiments in which acid was used subsequent to procaine there was a complete absence of inhibition (fig. 17*a*). But in 4 of 5 experiments, with the subsequent application of glucose, inhibition appeared of the same order as in experiments without prior application of procaine (fig. 17*b*).

5. *A Comparison of the Effects of Infusions of Acid, Glucose, Peptone and Fats into the Duodenum and into the Jejunum*

Twenty-six experiments were carried out on 2 dogs, one with an innervated gastric pouch, and the other with a denervated pouch. Each had cannulæ in both duodenum and jejunum.

The infusion into the *duodenum* of tap water (2 experiments), 20 per cent peptone (2 experiments), hydrochloric acid (3 experiments), 10 per cent dextran (2 experiments), 40 per cent glucose (4 experiments), and 50 per cent emulsion of ground-nut oil (2 experiments) resulted in inhibition of the secretory response to histamine in every case.



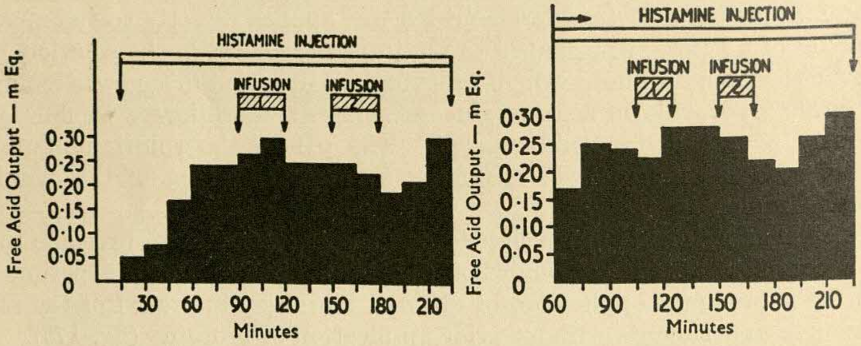
Pouch innervated

(*a*) Duodenal applications:
A1 = HCl pH 1·5
A2 = HCl pH 1·5
P = 5 per cent procaine

(*b*) Duodenal applications:
G1 = 20 per cent glucose
G2 = 20 per cent glucose
P = 5 per cent procaine

Fig. 17.—The inhibition of gastric secretion by low pH in the duodenum is mediated through a local neural mechanism in the duodenal mucosa which can be blocked by procaine. The inhibition by glucose is not dependent on a local neural mechanism.

The infusion of the same materials under the same conditions into the *jejunum* failed to produce inhibition in any of 13 experiments. On the other hand, clear *augmentation* of secretion was noted on each occasion that hydrochloric acid and glucose were infused into the jejunum, and on 2 of 3 occasions when peptone was infused (fig. 18).



Denervated pouch
Cannulæ in duodenum and jejunum
Infusions:
(1) glucose 40 per cent
30 ml. into jejunum
(2) glucose 40 per cent
30 ml. into duodenum

Innervated pouch
Cannulæ in duodenum and jejunum
Infusions:
(1) HCl pH 1·5
40 ml. into jejunum
(2) HCl pH 1·5
40 ml. into duodenum

Fig. 18.—The receptors of the small intestine mechanisms sensitive to pH and to osmolarity both lie in the duodenum.

6. *General Observations*

(a) *Post-inhibitory Rebound.*—In a number of experiments in which the response of gastric pouches to meat meals was inhibited by infusions of acid, glucose or fat, into the duodenum, and occasionally when histamine provided the stimulus, the period of inhibition was followed by one in which the output was much greater than that observed over a similar control period. The rise in output appeared to be invariably associated with an increased volume of secretion and only occasionally with increase in the concentration of the acid.

(b) *On a Critical Level of Osmolarity.*—Inhibition of gastric secretion seemed to appear when the osmolarity of the duodenal contents fell below, or rose above, a certain range. The production of inhibition, therefore, was usually a function of the total amount of solute introduced during the time of infusion (fig. 17).

Observations on the osmolarities of the infused solutions suggested that recognizable inhibition was obtained when a level of 275 milliosmols was reached and was marked when osmolarity rose above 425 milliosmols. Inhibition also appeared when the osmolarity of the infusate fell below 50 (tap water).

(c) *On the Appearance of Two Separate Mechanisms in the Duodenum Inhibiting Gastric Secretion.*—The more complete the destruction of the nervous connexions between the duodenum and the gastric pouch the less evident was the effect of *acid* in the duodenum on the secretory responses of the pouch. Such inhibition was slight and transient in simple denervated pouches and was only manifest in one-third of the experiments, and was totally absent from dogs in which either the antrum had been resected and the pouch denervated, or in which the pouches were autotransplanted. But solutions seeming to exert their effect through *osmolarity* were capable of inhibiting gastric secretion after either resection of the antrum or where the pouch was autotransplanted. Thus, intact nervous communications between the duodenum does not appear to be essential to the operation of the osmolar-sensitive mechanism.

(d) *On the Localization of the Receptor Mechanisms for Inhibiting Gastric Secretion.*—The evidence from the dogs equipped with cannulæ at two sites in the small intestine strongly favours the probability that both types of mechanism of inhibition which have been demonstrated are confined to the duodenum.

DISCUSSION

The results of this investigation confirm the many previous studies that hydrochloric acid, sugar and fats, placed in the duodenum of dogs inhibit gastric secretion. It was also confirmed that acid in the duodenum could inhibit the response to histamine and that all the other substances investigated, including hypertonic saline, sugar, fat and peptone, could do likewise. In view of the probable general rôle of histamine in the stimulation of acid secretion by whatever stimuli [Babkin, 1950; Sircus, 1953], this is not surprising, and the failure of previous authors is probably explained by their method of administering histamine in a single injection.

The possibility that osmotic factors played a part in the duodenal influence on gastric secretion was suggested by Day and Komarov [1939], and strong support for this suggestion is provided by the present results. Hypertonic saline, glucose, fructose, polysaccharide complexes and peptone were all shown to inhibit gastric secretion when introduced at concentrations above a certain level of osmolarity and, on the other hand, a less marked but recognizable inhibitory influence when hypotonic tap water was introduced. There is a notable similarity of inhibition produced by these different substances in different pouch and duodenal preparations in respect of latent period, duration of action and degree of effect. These observations, together with the evidence of the existence of a critical level of osmolarity necessary to produce inhibition, irrespective of the nature of the substance perfused, indicate that a single common osmoreceptor mechanism operates in the small intestine which is sensitive to different solutes. Furthermore, the evidence from experiments on dogs with autotransplanted pouches suggests that the osmoreceptor mechanism may release a humoral agent which influences the secretion from the gastric mucosa. However, it cannot be excluded that the mechanism may work by interference with the intimate vasculature of the gastric mucosa rather than directly with the secreting cells. An attempt to elucidate this was made by implantation of heated thermocouples directly into the secreting mucosa of the gastric pouch and recording temperature throughout the experiments. No alteration in temperature in the gastric mucosa was observed in three such experiments nor in others from simultaneously placed thermocouples in the lumen of the pouch, in the mammary gland, and in muscle, during, or subsequent to, the appearance of inhibition of secretion. However, there is little doubt that such methods are far too crude for the purpose and the only reliable way of estimating altered gastric mucosal blood flow would be by observations on the rate of diffusion of gases through the mucosa. No valid conclusions can thus be drawn from the thermocouple experiments.

The failure to block the excitation of the osmoreceptor mechanism by prior application of procaine would seem to suggest that it is not dependent on a local neuroreceptor in the mucosa but may be related to the absorption of the solutes. However, the existence of a critical level of osmolarity, and the ability of polysaccharide complexes to stimulate it do not support this. The failure to stop the excitation of the osmoreceptor mechanism by prior anæsthetization nevertheless serves to discriminate this mechanism from the one sensitive to changes of pH.

The ability of lowered intraduodenal pH to inhibit pouch secretion varied with the H-ion concentration of the duodenal contents, and the state of innervation of the pouch and duodenum. Thus in contradistinction to the findings of Gregory [1956] acid in the duodenum did *not* appear to influence the secretion from autotransplanted pouches. Likewise, gastric secretion was not inhibited by acid in the duodenum of dogs with denervated pouches and with the antrum resected. A slight and transient inhibition was obtained in a third of experiments in dogs with the antrum intact but the pouch denervated. The failure of Woodward *et al.* [1954] to obtain inhibition of meat-stimulated secretion by infusion of a duodenal fistula with N/10 HCl can be explained by their use of a denervated (Heidenhain) pouch preparation —and their conclusions are therefore invalid. The effect of antral resection may be to sever vagal connexions to the duodenal mucosa and so interfere more completely with the neural pathways of the mechanism than does the creation of a Heidenhain pouch alone, or possibly, to eliminate a contribution of inhibition from regurgitation of acid into the antrum. The fact that Code and Watkinson [1955] failed to observe inhibition *of any degree at all* in denervated pouches may be the result of the different method of instillation of acid employed. They used intermittent instillation over alternating 5-min. periods. In the promotion of secretion from gastric pouches more than one factor may be involved, one of which is the neural reflex which appears to be disturbed by acid in the duodenum. But other components of the excitation process may also be influenced by acid in the duodenum and this part would remain influenceable in the absence of the vagal innervation, and could be responsible for the moderate degree of inhibition observed in half the experiments on denervated pouches in this study.

That the effects were not due to regurgitation of acid into the antrum is shown by the effect of perfusing acid through the isolated duodenum in a dog with an innervated pouch (fig. 10 *d*).

These experiments have also confirmed that there exists a critical level of pH above which the mechanism is not stimulated. Pincus *et al.* [1944] found in dogs with cannulæ in the jejunum that secretion was depressed 50 per cent if the pH of the jejunum fell to 2·5, and 100 per cent if it fell to below 2·0. In this study it was observed that inhibition did not occur until the pH of the material in direct contact with the duodenal mucosa fell to 2·0 and that the site of action of the mechanism was restricted. It has been previously shown [Sircus, 1954] that acid in the small intestine can stimulate the secretion from gastric pouches, but this was observed in the perfusion of loops made up partly of duodenum and partly from jejunum; the similar findings by Code and Watkinson after 2 hours' instillation of acid into the duodenum may therefore be due to the onwards passage of the acid into the jejunum. Their paper shows that excitation of secretion did not appear during the first hour of instillation whereas *inhibition* during histamine stimulation does appear within the first hour; this supports the suggestion that the stimulation they observed originated in the jejunum.

Furthermore, the experiments in this study on the dogs with cannulæ in both duodenum and jejunum indicate clearly that the site of the inhibitor mechanisms sensitive to osmolarity and to pH lies in the duodenum. Fat, on the other hand, inhibited even when instilled into the *jejunum* but the possibility of upward diffusion or carriage of fat by antiperistaltic waves is not excluded.

As far as can be ascertained from the literature, all previous studies on the inhibition of gastric secretion by fat in the duodenum have been made on preparations with normal anatomical continuity in the small intestine. Hence, fat placed in the duodenum would have rapidly come into contact with enzyme-containing juices. The evidence from the experiments on the dog with the isolated duodenal loop indicates that prior digestion of fat is necessary before inhibition of gastric secretion is promoted from the duodenum. It is notable in this respect that the latent period before fats in the small intestine inhibit gastric secretion is usually 15–30 min.

Prior anæsthetization of the duodenal mucosa successfully blocked the effect of lowered pH, thereby demonstrating the dependence of this mechanism on local neural processes in the mucosa. Recently Iggo [1957] has demonstrated receptors in the gastric mucosa of cats which were stimulated to intense activity by contact with acid of low pH.

No previous studies of the effect on gastric secretion of protein materials in the duodenum have been found. Inhibition by the peptone solutions used in this study depended closely on the concentrations of the solute, but an unexplained observation was the virtual absence of inhibition when peptone was infused into the duodenum subsequent to excision of the antrum. This suggests that some of the inhibition noted with peptone solutions may be due to regurgitation from the duodenum but this is contrary to all known facts about the release of gastrin from the antral mucosa and so requires further study. That it could not be solely due to regurgitation into the antrum is demonstrated by the inhibition obtained on perfusing the isolated duodenal cap preparation with peptone.

ACKNOWLEDGMENT

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BOOK REVIEWS

THE PHYSIOLOGY OF NERVE CELLS. By J. C. Eccles. Baltimore: The Johns Hopkins Press. 1957. Pp. ix + 270. 30s.

There has recently been an unfortunate tendency for those working on the central nervous system to divide into synaptologists and others. On the one hand synaptologists have applied with more or less rigour the methods of the nerve physiologists to the motoneurone and have given the impression that this is the only intellectual activity worthy of the name of neurophysiology. Their excursions into problems of the organization of the nervous system as a whole have been less happy. They have been marked by considerable confidence, and supported by an apparent ignorance of the major nervous structures involved, so that even the nuclear masses of thalamus and basal ganglia have been dismissed as irrelevant to problems of cortical organization.

On the other hand organizational nerve physiologists have come to rely on the elegant generalizations of information theory, and control systems which though satisfying ways of describing behaviour are only rarely applicable in strictly mathematical terms and hardly ever referable to real anatomical structures.

Professor Eccles's latest book should help to heal this unnecessary division. His last chapter on aspects of functional architecture should be read first by organizational neurophysiologists. They will find it throws light on their basic problems with a wealth of illustration from structures of all parts of the vertebrate and invertebrate nervous systems. Such a reader will then turn with greater zest to the rather more difficult early chapters on the physico-chemical analysis of synaptic activity—chapters he will find both exacting and rewarding.

Although its field is so much more restricted, this is perhaps a more satisfying and impressive book than the *Neurophysiological Basis of Mind.* Recent work, very largely from Canberra, has provided data on motoneurones themselves in fields in which previously only analogies from muscle and nerve were available. No doubt there are many controversial points. The nature of the direct inhibitory pathway is still being questioned, and some may feel that dendrites of motoneurones may perhaps have some function besides that of pumping out sodium and absorbing potassium ions.

Nevertheless this book provides the clearest and most complete account of the variety of devices developed by the nervous system for the transmission of impulses, and provides a solid basis on which to build schemes of neural organization.

D. WHITTERIDGE.

PROGRESS IN BIOPHYSICS AND BIOPHYSICAL CHEMISTRY. Vol. 7. Edited by J. A. V. BUTLER and B. KATZ. London: Pergamon Press, Ltd. 1957. Pp. viii + 362. £3, 10s.

The first problem facing the editors of this series of reviews is evidently to define the field covered by "Biophysics and Biophysical Chemistry". Like their predecessors, Professor Butler and Professor Katz have adopted a liberal interpretation, and their choice of topics should satisfy the most catholic appetite. However, the variety of the disciplines that can legitimately be classified under the general heading of biophysics, sets the authors of the individual articles a difficult task. If more than just one or two of the contributions are to be acceptable to any given reader, the writers must aim not only at satisfying specialists in their own field, but also at providing non-specialists with a concise account of recent progress in the field in question, and of its implications in a wider sphere. After attempting to read right through the volume, one is left with an impression that the first of these objectives is easier to achieve than the second.

The volume opens with a survey by Dr. Grundfest on the physiology of the electric organ of fishes and its relation to general electrophysiology. This is certainly the most complete account of the discharge mechanism of the electric organ to be published in any language for many years, but in places the wealth of detail is such that it seems liable to defeat all but the most assiduous electrophysiologists. It is also a pity that the editors have permitted the inclusion of such copious footnotes; these cannot be ignored, for they incorporate some of the author's most illuminating remarks, but they do not make for easy reading. The second contribution is by Professor and Mme Wurmser, who write about the thermodynamics of isohæmagglutinin reactions. They have successfully overcome the handicap of a language foreign to them, and give an interesting though somewhat specialized account, mainly concerned with their own work on the subject. There follow two articles on biological applications of radioactive tracers. Dr. McFarlane writes lucidly on the use of labelled plasma proteins in studying nutritional problems, and makes a number of points which are relevant in interpreting the results of tracer experiments in other fields. Dr. Arnstein and Dr. Grant discuss the use of isotopes in the study of intermediary metabolism, but somehow, perhaps because of the extreme complexity of the subject, do not entirely succeed in making clear just what advances in biochemistry have resulted from an exploitation of tracer techniques. After a clear and critical account of the X-ray crystal analysis of bone by Dr. Clark and Dr. Iball, comes what is probably the most important contribution to the volume—Mr. A. F. Huxley's discussion of muscle structure and theories of muscular contraction. This consists in a historical review of some of the earlier theories, an account of recent research on muscle structure, and a new hypothesis for the mechanism of contraction which appears here in print for the first time, and which attempts to reconcile not only the structural and chemical evidence, but also the mechanical and thermal data. Mr. Huxley's presentation of a difficult subject is a model of clarity and forceful thinking. The final article is by a botanist, Dr. Whittingham, on chloroplast structure and energy conversion in photosynthesis. It seems to leave a number of questions unanswered, but this is presumably the fault not of the author, but of the present state of research in this field.

The book is admirably produced, and the editors are to be congratulated on maintaining the high standard set by previous volumes in the series.

R. D. KEYNES.

THE LIFE OF MAMMALS. By J. Z. YOUNG. Oxford: Clarendon Press. 1957. Pp. xv + 820. 84s.

In composing *The Life of Mammals*, Professor J. Z. Young has intended that it should be a systematic aid and companion book for practical courses of anatomy and embryology. The book is wide in scope. The first two-thirds deal with both morphology and function of the adult animal while the final section is a very considerable essay in embryology. It is nevertheless rather shorter than many standard texts dealing with anatomy or physiology alone. Principles rather than detail are considered and economy has been achieved by avoiding extensive discussion of more obscure phenomena and by adroit use of well produced illustrations. Some of these are diagrams explanatory of ideas or principles; others illustrate points of structure. Each is well-annotated so that the meaning is clear.

This is not a book about which there will be unanimity of opinion. It is open to serious criticisms but it also has virtues which a teacher in the biological sciences may well feel outweigh any possible defects. Firstly it is extremely readable and the ideas which it contains have from some points of view an attractive simplicity. Secondly it offers a rapid birds' eye view of our present knowledge of the structure and functions of mammals as a class. This is coupled with a continuity from one chapter to the next that is so lacking from most modern texts. For each topic the information from experiment and observation is selected and always kept subordinate to the explanation of function. One can indeed detect a certain impatience with phenomena that cannot be rationalized in this way. And perhaps it is for the same reason that in this work the theoretical framework is provided by the broadest generalizations of information theory. It is this, what one may call the philosophy of the book, which must give rise to some misgivings about its value as a text.

The reason professed by the author for the use of information theory is that "The language . . . [of information theory] . . . provides the possibility of unifying the different treatments that are adopted by anatomy, physiology, embryology", etc., and elsewhere he implies that the application of this language to biological phenomena makes these more meaningful. However, in the final analysis information theory is based on ideas drawn from man-made machinery and models and we may question, whether what is in effect model-thinking, is necessarily the most useful means of understanding living processes. Professor Young appears to think that it is; but others may be less certain.

This method of thinking clearly has been of great value in some problems and for some purposes. But to try and comprehend all living processes within such a scheme can lead to near absurdities, as for example, "we can consider . . . the process of embryological development as one of communication" or in the description of the laying down of fat depots as an "obvious example of predictive action by the body". Again there is oversimplification in describing the gene solely as a carrier of information. Either new meanings must be attached to such words as information, prediction, determination, etc., or other aspects of complex biological entities must be ignored.

More serious criticism is that the greater part of the theoretical treatment hinges on teleological arguments. The desire to explain biological events in terms of purpose is human and most understandable; many respectable biologists indulge in such speculation on occasion, perhaps very frequently, and find it stimulating and helpful; but to place such reliance on teleology as is done here reveals a naïvete which is out of keeping with present scientific knowledge. Indeed in this respect the book is reminiscent of some scientific writings of the late nineteenth century when to some it appeared that all living processes would eventually yield to simple rational explanations derived from a consideration of the survival needs of the organism.

If as I have suggested *The Life of Mammals* errs in fundamental doctrine, I can nevertheless recommend it to others for it is fresh and vigorous, stimulating and provoking; and informed throughout with the author's wide knowledge and scholarship.

CATHERINE HEBB.

THE CHEMICAL BASIS OF HEREDITY. Edited by WILLIAM D. MCELROY and BENTLEY GLASS. Baltimore: The Johns Hopkins Press. 1957. Pp. vii + 848. £5.

This book records the proceedings of a symposium organized by the McCollum-Pratt Institute of Johns Hopkins University, which took place in the summer of 1956. There has recently been rather a spate of collaborative books of this kind, but the present one is a very good example of the genus. It deals with the most fundamental problem of analytical biology—the chemical nature and functioning of the basic units on which biological organisms are based. The contributors are mainly, though not entirely, American, and are of the very highest standard, and they have been given adequate space for a full exposition of their views. The recording of the animated discussions which followed some of the papers is very well managed, and adds considerably to the value of the work. The presentation is in general at a rather highly technical level, but all workers in the large field of chromosomes, genes, nucleic acids and viruses will find the book essential.

C. H. WADDINGTON.

OPINIONS AND CRITICAL JUDGMENTS ON PROFESSOR G. P. ARCIERI'S BOOK, *The Circulation of the Blood and Andrea Cesalpino of Arezzo.* Preface by Dr. N. PENDE. New York: Alcmaeon Publications. 1957. Pp. 27.

The Harvey centenary of June 1957 has been celebrated by a pamphlet reiterating the main conclusions of Professor G. P. Arcieri who published in 1945 a book suggesting that Cesalpino deserved the credit for the first understanding of the circulation of the blood.

The quotations on which Professor Arcieri relies are substantially those quoted by the learned medical historian H. P. Bayon (Annals of Science, 1938, **4**, No. 4). Arcieri does not, however, quote a passage from the Praxis Universæ Medica by Cesalpino published in the year of his death (1603) "Distribuitur sanguinis in quatuor venas scilicet cavam et aortam, arterialem venalem et venam arteriosam-totum corpus irrigat instar quatuor fluminum ex Paradiso prodeuntium."

"The blood is distributed by four veins, namely the cava and the aorta, the arterial vein and the venous artery—it irrigates the whole body in like manner as the four rivers issuing from Paradise."

This is hardly a simile which would occur to anyone convinced of the motion of the blood in a circle.

D. W. WHITTERIDGE.

BOOK NOTICES

ESSENTIALS OF FLUID BALANCE. By D. A. K. Black. Oxford: Blackwell Scientific Publications. 1957. Pp. xiv + 127. 18s. 6d. This book deals with the distribution of body water, the regulation of the sodium and potassium and of the pH of the body fluids and the disorders of these factors that are seen clinically. Any consideration of the fluid and electrolytes of the body must inevitably be strictly quantitative; many medical men never come to grips with the subject because they are frightened by such quantities as "millimols" or "glomerular filtration rate". Dr. Black's work gently and lucidly leads those who have been perplexed and confused through the intricacies of his subject. He makes frequent reference to the experimental background of the clinical investigation; his bibliography is a careful selection of—and guide to—the enormous literature on fluid and electrolyte balance.

THE CONCEPT OF DEVELOPMENT; AN ISSUE IN THE STUDY OF HUMAN BEHAVIOUR. Edited by Dale B. Harris. Minneapolis: University of Minnesota Press. 1957. Pp. 287. £1, 18s. In December 1955 the Institute of Child Welfare organized a conference to consider the general concept of development as it is used in biology, and in particular its applications to the study of behaviour. This volume records the proceedings. Much of it falls outside the field of physiology, into the domains of philosophy, psychology and the social sciences, but there are several interesting articles on the physical and behavioural changes associated with growth and development in man and other animals.

THE CARBOHYDRATES. CHEMISTRY, BIOCHEMISTRY, PHYSIOLOGY. Edited by Ward Pigman. New York and London: Academic Books Ltd. Pp. xvii + 902. £6, 8s. 6d. It is strange to find a book on the physiology of carbohydrates with no reference to insulin in the index. The physiology section is limited to thirty-five pages on carbohydrates in nutrition. Indeed the word "physiology" in the title is misleading and should have been deleted. However the book gives a full account of the chemistry of the carbohydrates and of the enzyme systems concerned with their biological transformations. As such it should prove a valuable reference work for biologists.

INTRODUCTION TO GENERAL EMBRYOLOGY. By A. M. Dalcq. Oxford: Clarendon Press. 1957. Pp. vii + 173. £1, 5s. This book stems from thirteen broadcast talks which the author, who is Professor of Human Embryology at the University of Brussels, gave over the *Université Radiophonique* of Paris. Even a third programme enthusiast will find them hard going, but the effort is well worth while, especially for those whose student embryology is a long way distant. As one read on, the conclusions grew that embryology is the queen of the biological sciences from which all our problems stem and one was grateful to Professor Dalcq for reminding us of so much that is fundamental. He has added some excellent diagrams and microphotographs and one beautiful coloured plate, all of which facilitate understanding. Jean Medawar has made a competent translation and the Clarendon Press has contributed their part to a book which was a pleasure to read.

HORMONES IN BLOOD. Ciba Foundation Colloquia on Endocrinology. Vol. 11. Edited by G. E. W. Wolstenholme and Elaine C. P. Millar. London: J. & A. Churchill, Ltd. Pp. vi + 416. 56s. Here is another publication reporting one of the stimulating and altogether pleasant Ciba Colloquia. The recognition and assessment of hormones in blood is both important and difficult. Blood is the means of transport of the hormones, all of which exist in it simultaneously in minute amounts with some free and other bonded to larger molecules. The separation of these substances from each other is a necessary preliminary for discovering at what rate they are liberated or produced and how their concentration and balance in relation to each other varies in different states of health and disease. A large number of the communications are devoted to the adrenal steroids and deal with such aspects as their concentration in scurvy, their physico-chemical state in the blood, the cortico-steroid releasing activity of blood, and changes in the gland itself in relation to the concentration of steroids in the efferent veins of the gland. The thyroid hormones were similarly treated in full. Single communications given on the neuro-hypophysial hormones, œstrogen, progesterone and insulin. For anyone familiar with the subjects the discussions are often as illuminating and helpful as the prepared papers, for in these various workers voice their doubts and present suggestions. Once more we have cause to be grateful to the Ciba Foundation.

METHODOLOGY OF THE STUDY OF AGEING. Ciba Foundation Colloquia on Ageing. Vol. 3. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. London: J. &. A. Churchill, Ltd. Pp. x + 202. 32s. 6d. The methodology of ageing is discussed in relation to widely different systems. In this way papers are presented on such diverse subjects as ageing in cells, the use of inbred strains in animals, nutrition and ageing in Africans and processes of ageing as seen in intelligence and emotion. This book will be of value in showing how wide an attack is being made on the problem. It is difficult to get any synthesis of the subject from such a scrappy treatment. The records of the discussions that took place are unusually helpful.

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REVIEW ARTICLE

FATTY DEPOSITION: A DISORDER OF THE CELL. By KENDAL C. DIXON. From the Department of Pathology, University of Cambridge.

(*Received for publication* 14*th October* 1957)

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INTRODUCTION

THE appearance of droplets of liquid fat in the cytoplasm is a most prominent feature of disordered cells. In health such droplets are seldom visible, and cannot usually be demonstrated in the cells of kidney, liver and heart muscle; the fat in these tissues is not in liquid form capable of dissolving the dyestuffs of the Sudan group. This invisible or concealed fat can, however, be extracted from the tissues with mixtures of lipid-solvents. The lipocytes of areolar tissue, on the other hand, contain droplets of liquid fat which have the capacity to dissolve the Sudan dyes; this visible fat displays the properties of a liquid, which forms a separate phase from the rest of the cytoplasm.

Cells, which in health are devoid of visible fat, may in disease contain spherical droplets of visible fat in abundance; this fat, like that of the lipocytes, is in liquid form and is readily detected with the Sudan dyes. Recent advances in colloid science, and also in knowledge of the ultrastructure of cells [see Sjöstrand, 1956] have enabled a picture of the disposition of cellular fat to emerge. This picture, although still highly imperfect, permits a preliminary survey of the kind of mechanisms involved in fatty deposition.

For long a controversy centred round the question, whether the deposition of fat in damaged cells depends on the unmasking of previously concealed lipid, or on an accumulation of excess fat. The ensuing pages show that both these processes may contribute to the deposition of visible droplets of fat in disordered cells. Moreover, the actual accumulation of fat may be a sequel as well as a cause of the deposition of liquid fat. The divergence between the concepts of accumulation and unmasking is thus more apparent than real. The fact that a quantitative increase in fat so frequently accompanies fatty deposition is well established [Dible, 1934; Dible and Gerrard, 1938; Popjak, 1945]. Early views on fatty change, including those of Virchow, are well summarized by Wells [1925] and Cameron [1952].

The word deposition is here used to describe the appearance of visible droplets of liquid fat and cholesterol esters in damaged cells. The optical properties of these droplets are such as to confer a yellowish white or "fatty" appearance to the organs which contain them. Deposition is a purely descriptive term, which carries no implication of underlying cause. Deposition neither implies unmasking of previously invisible and concealed lipid nor the accumulation of excess of fat, as the basis for an essentially visual phenomenon. The words degeneration, infiltration and accumulation are often employed; these words have causal connotations, each of which unfortunately implies that one kind of mechanism is responsible for the appearance of visible fat. The use of such words, which inherently postulate a certain type of mechanism, has moreover deflected attention from the basic problem of how fat becomes visible. Our prime concern is, therefore, the mechanisms responsible for the deposition of visible droplets of liquid fat in dead, dying and disordered cells.

STATE OF INTRACELLULAR FAT

The orientation and arrangement of triglyceride molecules in the cell must be connected with the presence of emulsifying and stabilizing colloidal electrolytes such as the phospholipids. Fats and oils are not soluble in water, but in the cell, are frequently so finely dispersed as to be invisible by microscopic methods. The capacity of protoplasm to hold neutral triglycerides in such a condition of dispersion depends probably on the presence of colloidal electrolytes of the type classified as "paraffin-chain-salts" or "soaps" [Alexander and Johnson, 1951]. These compounds are potent emulsifying agents. In general they contain a small ionized or polar group with a high affinity for water (X in fig. 1) and a large unionized non-polar portion (Y in fig. 1) with no affinity for water.

Phospholipids, which are constituents of all cells belong to this class of colloid. Lecithin, for instance, has both positive and negative ionizable groups in its polar portion; but one of these is likely to predominate at any particular pH to give a net positive or negative charge to the polar end of the molecule.

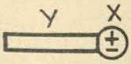

FIG. 1.

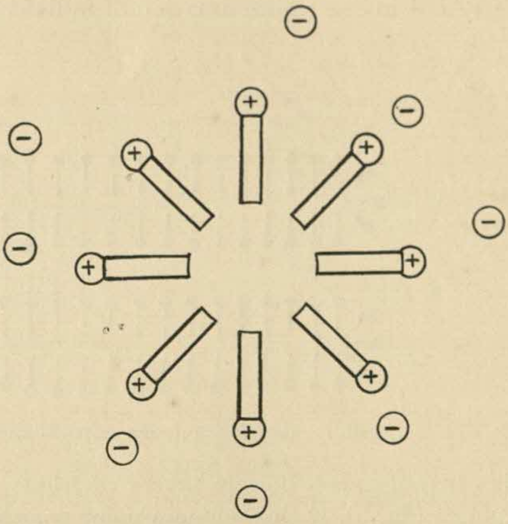

FIG. 2.—Spherical micelle.

If molecules of this type of paraffin-chain-salt existed in aqueous solution as single molecules with exposed hydrocarbon chains, relatively enormous interfacial forces between the hydrocarbon portions of the molecules (Y in fig. 1) and the surrounding water would have to be overcome; the tendency to aggregation of such molecules would be overwhelming. Advances in colloid science [see Alexander and Johnson] have established that in dilute solution these substances exist as spherical micelles, in which the polar group with strong affinity for water is alone in contact with the aqueous phase and the non-polar hydrocarbon portion is orientated so as to have no contiguity with the surrounding water (fig. 2).

In aggregates like those shown in fig. 2, the hydrocarbon-water interface is at a minimum so that the free energy of the system is at its lowest. Aggregation to this kind of spherical micelle accounts for the stability of colloidal electrolytes in aqueous solution.

With increase in concentration of colloidal electrolytes these spherical micelles approach each other. As the distance between the charged micelles is diminished, their mutual repulsion progressively increases, until, with high concentrations, relatively enormous repulsive forces would have to be countered if this spherical micellar arrangement were to persist. Thus a high concentration of paraffin-chain-salts would favour other types of arrangement, so as to avoid compression of the spherical micelles. One such arrangement is the laminar micelle, first proposed for colloidal solutions by Hess and Gundermann [1937]; these workers, from observations on X-ray diffraction shown by concentrated solutions of sodium oleate, concluded that paraffin-chain-salts can become arranged in laminar leaflets ("blätchen-förmigen Micele") orientated in parallel with one another. This parallel arrangement of laminar leaflets permits an immense increase of micellar substance in solution without undue approximation of individual micelles (fig. 3).

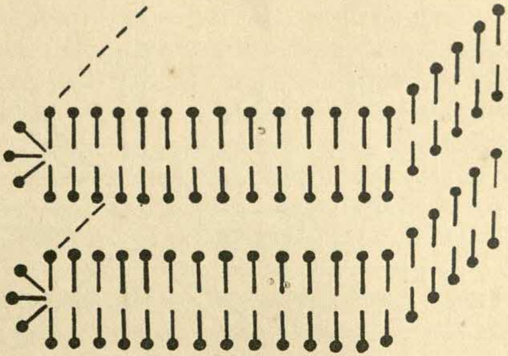

FIG. 3.—Laminar micelles in parallel orientation.

In this arrangement double layers of lipid molecules are orientated in parallel plates (fig. 3), with their non-polar ends internal and thus separated from the aqueous phase, and with their polar ends external and in contact with water. Thus many more molecules can be accommodated in the same space than when disposed as spherical micelles with the same intermicellar separation.

It is highly probable that such laminar micelles play a prominent part in cell structure. Hartley [1948] expressed this view in a specially cogent manner—"this picture of separated laminæ has frequently been described and equally frequently drawn. The difficulty is to stop drawing it, and one may fairly ask whether Nature would not find a corresponding difficulty".

The properties of colloidal electrolytes which lead to the formation of laminar micelles may determine the laminar structure of various cellular components. Leathes [1925] emphasized the tendency of phospholipids to form extensive interfaces with water and suggested that this property may be of importance in the structure of cell membranes. Danielli [1936, 1951; see also Danielli and Davson, 1935] postulated a bimolecular lipid micelle with external polar groups and adherent films of protein as the basis of the cell membrane. Recent studies on the ultrastructure of cells by use of the electron microscope indicate the possibility of a similar arrangement for the surfaces and cristæ of the mitochondria [Sjöstrand, 1956]. Moreover, flattened laminar sacules, orientated in parallel, constitute the ergastoplasm or endoplasmic reticulum; these may also consist of laminar micelles of lipid along with adherent protein.

Now a remarkable property of such micelles is their power to incorporate fats and oils, which are themselves insoluble in water, and so enable these insoluble substances to be held in a stable emulsion in water [see Alexander and Johnson]. In the living cell it is likely that fat molecules are occluded inside spherical, cylindrical and laminar micelles of phospholipid so that the fat is not in direct contact with water. If phospholipids are present in relatively large amounts, the micelles would mainly be laminar in type with some fat molecules held between the double layers (fig. 4). While, if the phospholipids are in relatively small amount, large spherical micelles would predominate, in which each fat droplet is covered by a unimolecular layer of orientated phospholipid (fig. 5).

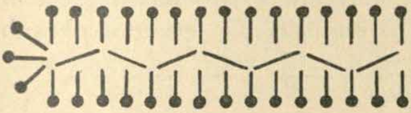

FIG. 4.—Fat molecules in laminar micelle] of phospholipid.

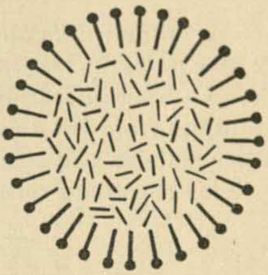

FIG. 5.—Spherical globule of fat covered by film of phospholipid.

We may thus recognize two kinds of disposition of cellular fat:

(i) Fat may be enclosed in laminar (or cylindrical) micelles as in fig. 4. This may be the normal condition of intramitochondrial fat. Fat molecules dispersed in this way are not aggregated as liquid droplets. This micellar fat is not visible as spherical droplets nor can it dissolve non-polar dyes like Sudan IV; it has neither the optical nor the solvent properties of liquid fat, is invisible by microscopic methods and is essentially masked or concealed fat. In the ensuing pages this will be referred to as *micellar fat*.

(ii) On the other hand cellular fat may be held as spherical aggregates of liquid fat covered by a unimolecular film of phospholipid (fig. 5). This fat, except for minimal amounts adsorbed in the surface films, is present as liquid spherical globules. It can dissolve non-polar dyes like Sudan IV and has the optical properties of liquid fat. This fat is not masked by micellar dispersion and is plainly visible by microscopy; it also gives the tissues a creamy or fatty appearance. It may conveniently be termed globular fat.

The ratio of fat to phospholipid plays a dominant part in determining whether globular fat occurs or not. Excess of fat beyond the amount which can be held in laminar micelles will cause globular fat to be deposited. Similarly a lack of phospholipid will promote the same transition from micellar to globular fat. As soon as this visible fat is deposited there exists an equilibrium between it and the invisible micellar fat (fig. 6).

$$\underset{\text{not visible}}{\text{MICELLAR FAT}} \;\underset{\text{phospholipid; protein}}{\overset{\text{cholesterol}}{\rightleftarrows}}\; \underset{\text{visible}}{\text{GLOBULAR FAT}}$$

FIG. 6.

In many normal cells the equilibrium shown in fig. 6 is not operating, since all the fat is in the invisible micellar state. Once the threshold for deposition is surpassed, either excess of fat or lack of phospholipid will push the equilibrium from left to right depositing visible droplets.

Another cellular constituent which may affect this equilibrium is cholesterol. This substance antagonizes the emulsifying action of phospholipid [Seifriz, 1923; Corran and Lewis, 1924] and thus is likely to impede micellar dispersion (fig. 6). How far protein takes part in the dispersion of fat is uncertain; but it is likely that protein is adherent to micellar surfaces in cells [Danielli and Harvey, 1935], and it may also contribute to the formation of lipoprotein complexes which are not visible as globular fat. Much of the lipid of blood is now known to be associated with protein.

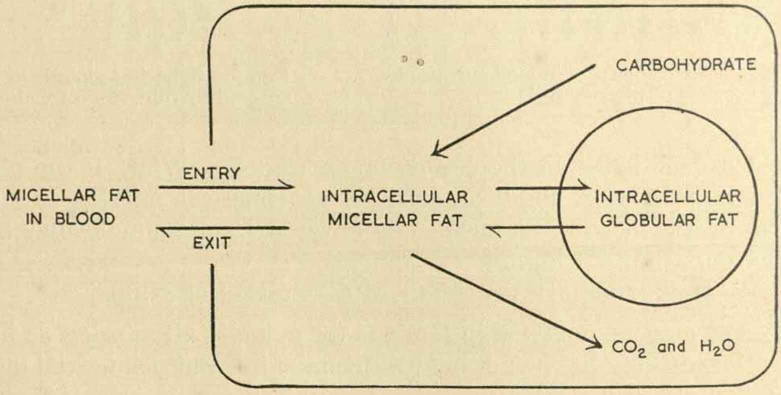

FIG. 7.—Intracellular relation between micellar and globular fat.

Globular and micellar fat probably differ greatly in their cellular reactivity. Visible globular fat is outside the zone of active metabolism and transport (fig. 7). Micellar fat, on the other hand, is close to enzymes which may be adherent to micellar surfaces. The oxidation of fat is known to occur inside the mitochondria where fat is dispersed in micellar state. Micellar fat, stabilized by phospholipid (and possibly forming a lipoprotein complex) may also be the form in which fat is transported from the cell. Similarly fat, formed in the cell from carbohydrate or entering the cell as preformed fat, is almost certainly at first located in micellar structures. This dominant position of micellar fat is indicated in fig. 7.

The researches of Schoenheimer and his colleagues [see Schoenheimer, 1946] demonstrated by use of isotopes that even globular fat of the depôts is continuously being synthesized and degraded. This lability of globular fat depends on its equilibrium with micellar fat, into which it is converted for metabolism and transport (fig. 7).

MECHANISM OF FATTY DEPOSITION IN DISEASE

In the healthy cells of parenchymatous organs neutral fat is normally held in micelles and is invisible. Probably visible droplets of lipid are not formed in cells unless a certain ratio of fat to phospholipid is exceeded. Once this ratio is surpassed *either* by diminution in phospholipid *or* by excess of fat, then visible globules of fat are deposited.

Deposition of visible droplets of fat in the cell may thus arise by:

(i) Conversion of previously invisible micellar,* mitochondrial or complex lipid to visible droplets of fat, without any initial increase in the total content of lipid in the cell. In this way equilibrium between micellar and globular fat is established so that visible globular fat is formed. This process generally depends on diminution in the quantity of phospholipid or alteration in its capacity to promote the formation of laminar micelles.

(ii) Absolute increase in the quantity of cellular fat to an extent which can no longer completely be accommodated by micelles so that visible droplets are deposited. Substantial increase in cellular fat, without corresponding increase in phospholipid, enables the equilibrium between micellar and globular fat to be reached. Above this threshold excess of fat results in more and more visible droplets being deposited.

The first of these processes is often called phanerosis (φανερός = clear, apparent). This word, when used in this sense, is unsuitable, because fat becomes visible or apparent in all examples of fatty change.

The second process is sometimes termed accumulation. This usage is equally undesirable, since accumulation means piling up, but does not imply the appearance of visible droplets. The use of the word accumulation should therefore be restricted to quantitative increase in fat.

The deposition of fat, whether primarily caused by impairment of dispersion, without increase in fat, or by accumulation, with a large initial excess, depends on the same transition from invisible to visible fat. Moreover, only micellar fat is likely to take part in katabolism and transport. The transition of micellar to globular fat would therefore impede the rate of removal of fat from the cell and so result in accumulation of more fat in the relatively inert globular form. This transition, even when not caused by initial excess of fat, will thus promote accumulation by interfering with utilization. Accordingly conversion of micellar to globular fat, without initial increase in fat, will tend to produce a secondary surplus of cellular fat, because liquid droplets cannot directly be utilized. Thus, irrespective of its initial cause, deposition of globular fat is likely to be accompanied by ultimate increase in the content of fat in the cell.

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* Masson [1956] considers that fat masked in "micelles vivantes" becomes apparent as droplets in fatty degeneration.

The argument, which has long centred round the question whether or not accumulation is a necessary concomitant of visible deposition, accordingly loses much of its significance. The solution of this argument does not provide an unequivocal discrimination between unmasking and accumulation as *initial* mechanisms for the appearance of fat in damaged cells. However, in view of the continued interest in the quantitative changes which accompany fatty deposition, a brief outline is given of some recent evidence on this question.

DEPOSITION WITHOUT ACCUMULATION

At one time deposition without accumulation ("phanerosis") was believed to occur in many diseased tissues. After accumulation had effectively been demonstrated in damaged liver, it was still held that visible fat may appear in cardiac muscle and renal tubules without increase in the cellular content of fat. The careful work of Dible on heart muscle and of Popjak on kidney (see below) established that quantitative increase also accompanies visible deposition in both these tissues.

Best, Hartcroft, Lucas and Ridout [1955], however, have reopened the question; in the liver of rats, insufficiently supplied with protein, they found that droplets of fat may become visible without increase in fat. This change may be due to the liberation of fat from a lipoprotein complex which decomposes owing to lack of protein; this process may be regarded as equivalent to the conversion of micellar to globular fat without any accumulation of fat. Dispersion in the micellar state may well depend on stabilization by protein as well as by phospholipid, so that lack of protein may result in aggregation of liquid droplets.

It is also possible that visible liquid lipid may arise in the cell by disintegration of complex lipids, which are themselves invisible or at least not in the form of spherical droplets. Wallerian degeneration of myelinated nerve fibres affords an example of such a process.

Wallerian Degeneration.—When a myelinated nerve fibre is divided, the myelin in the peripheral portion at first disintegrates into elipsoidal and ovoidal masses containing a central plasmatic space. Later these elipsoids and ovoids are transformed into homogeneous droplets and granules, which have a descending order of size and are devoid of central spaces.

Normal myelin, although stainable by Sudan black, is practically unstained by Sudan III and IV. It is birefringent, and its lipids are held in a crystalline lamellar lipoprotein complex and not as liquid droplets [see Wolman, 1956 *a*; Finean and Elkes, 1955]. Myelin contains three principal lipids, namely cerebroside, sphingomyelin and free cholesterol [Johnson, McNabb and Rossiter, 1948, 1949 *a*] and also phosphatidyl serine and acetal phosphatide [Brante, 1949]; little or no cholesterol ester and no neutral fat, however, are present among its lipids. Myelin stains well with the periodic acid-Schiff method (possibly due to galactose in cerebrosides and to unsaturated fatty acids); it also gives the plasmal reaction for acetal phosphatide and Schultz's reaction for cholesterol [Noback and Montagna, 1952].

When myelin degenerates following the section of nerves, cerebroside, sphingomyelin and free cholesterol are at first little changed and then later rapidly diminish; there is some increase of cholesterol esters and a late increase in neutral fat, while the total lipids steadily diminish [Johnson, McNabb and Rossiter, 1949 *b*]. Degenerating myelin rapidly loses birefringence and also attains the capacity (not possessed by normal myelin) to stain with Sudan III [see Prickett and Stevens, 1939].

The cytochemical changes in degenerating myelin were thoroughly studied by Noback and Montagna [1952]. The elipsoinds and ovoids, which are first formed, have all the properties of normal myelin. But the final granules do not stain with either the periodic acid-Schiff or plasmal methods, and are no longer birefringent. Moreover both the droplets and granules stain with Sudan IV (Sudano-positive stage of Spielmeyer).

These granules of lipid which appear during the course of Wallerian degeneration have thus developed the properties of liquid fat and have lost the chemical and physical properties of myelin. The appearance of these lipoid granules among the products of degeneration of myelin cannot be due to the accumulation of lipid, since the total lipid is diminished. This may fairly be regarded as an example of unmasking without accumulation. Whether this process involves disintegration of complex lipids with liberation of neutral fat or merely the conversion of a complex micellar structure to liquid droplets is uncertain.

Formerly the appearance of Marchi-positive droplets during Wallerian degeneration was also adduced as evidence for the formation of unsaturated fat from degenerating myelin. Wolman [1956 *a*, *b*], however, showed that unsaturated fats are probably not responsible for the Marchi reaction, but that this may depend on the liberation of a polysaccharide during the process of disintegration.

Deposition with Accumulation

Excess of fat in the cell may be a cause of deposition of visible droplets. Moreover, even when other factors are concerned in the first place with initiating deposition, accumulation of an excess of fat, as we have seen, is likely to follow the appearance of visible droplets as a secondary sequel. Much work has, however, been devoted to demonstrating that actual accumulation of fat accompanies the appearance of globular fat in disordered cells. Only some of the recent evidence on this question is considered here; the earlier literature is summarized by Cameron [1952] and by Wells [1925].

Liver.—Barret, Best and Ridout [1938] showed that starvation, injections of anterior pituitary extract, and poisoning by carbon tetrachloride all cause gross accumulation of excess of fat in the livers of mice fattened by linseed oil which had previously been hydrogenated with deuterium. In this elegant series of experiments the fat labelled with deuterium was first deposited in the depôts and later mobilized so as to enter the liver cells. The fat extracted from the liver was oxidized to water and CO_2, and the content of the deuterium in the fat was then estimated by measurement of the specific gravity of the water formed on combustion. This work showed in a novel manner that deposition of fat in damaged liver cells is accompanied by accumulation of excess of fat which has migrated from the depôts.

Heart.—In heart muscle deposition of fat is more prominent in fibres close to the cavities than in the more superficial regions. Dible [1934] established that the total amount of fat in these inner portions which contain visible droplets of fat is greater than in the peripherally situated muscle which does not show deposition. Fatty deposition in cardiac muscle is thus associated with quantitative increase in fat [see also Dible and Gerrard, 1938].

Kidney.—At one time fatty deposition in damaged kidney cells was regarded as an example of phanerosis without accumulation of excess fat. Although injured renal cells may contain copious visible droplets of fat, the detection of an increase in extractable fat for long eluded estimation. This difficulty may arise from the fact that only some of the cells are affected, so that the gross increase in extractable fat in the whole kidney is very small. Popjak [1945], however observed that visible deposition of fat is more frequent in the medulla than in the cortex, and that, if independent analyses are made on the zones of the kidney seen to be affected, then an increase in extractable fat can be detected. Thus deposition appears to be accompanied by a definite accumulation of fat.

It is thus clear that the deposition of visible fat in damaged cells is frequently accompanied by accumulation of fat in excess. This may equally be an effect as well as a cause of the appearance of visible droplets of fat, and so provides no unambiguous evidence on the mechanism of fatty deposition.

CELLULAR DETERMINANTS OF DEPOSITION

The intracellular deposition of fat depends, as we have seen, on the conversion of micellar to globular fat. Two basic mechanisms may be responsible for this change:

A. Interference with the micellar or mitochondrial dispersion so that invisible dispersed fat is aggregated into liquid droplets; this must involve disintegration of the structures which retain cellular fat in its normally invisible state.

B. Increase of intracellular fat to an extent which cannot be held in the micelles, so that the excess is deposited as visible fat.

We will now consider the kinds of derangement within the cell which may bring these two mechanisms into operation.

A. *Interference with Micellar Dispersion*

1. *Lack of phospholipid.*—Lack of phospholipid appears to be one of the most important single factors in disrupting micellar structure so as to liberate globular fat. Much micellar fat may be located inside mitochondria which are probably micellar structures themselves [see Sjöstrand, 1956]; Dawson [1957] suggested that phospholipid may be responsible for maintaining the integrity of mitochondria. Phospholipids are, moreover, the principal paraffin-chain-salts available within the cell for promoting the formation of micelles. Emulsions of fat *in vitro*, when stabilized by phospholipid, become turbid if the phospholipid is destroyed; this effect is seen when the stabilizing lecithin in an emulsion of egg yolk is destroyed by the α-toxin of *Clostridium welchii* [MacFarlane and Knight, 1941]. Similarly, without sufficient phospholipid, intracellular fat may become aggregated and globules make their appearance. Such globules probably cannot be utilized either by mobilization away from the cell or by oxidation within it; globular fat thus remains as a relatively inert residue, which can only re-enter metabolic pathways by becoming re-dispersed in the micellar state. Accordingly accumulation of excess of fat, in addition to appearance of visible droplets, may be a sequel to the action of lack of phospholipid in preventing micellar dispersion.

2. *Lack of choline and inositol.*—The synthesis of phospholipid is a delicate process which may be impeded in various ways. Lack of choline and lack of inositol deprive the cell of essential building stones for fabrication of phospholipids containing choline and inositol. Deficiencies in both cause deposition and accumulation of fat in the liver [see Best, Lucas, Patterson and Ridout, 1946]. Welch [1936] proved that choline is indeed a precursor of phospholipid; he fed arsenocholine to animals deficient in choline and showed that this is followed by the entry of arsenic into the phospholipids of the tissues. Lack of choline can thus interrupt the formation of phospholipids and so impede micellar dispersion of fat. Deposition of globular fat along with defective utilization are sequels to interference with micellar dispersion. Lack of choline, in addition to causing deposition of fat in fibres of heart muscle, may also kill the fibres [Wilgram, Hartcroft and Best, 1954]; it seems likely that lack of phospholipid is responsible for necrosis as well as fatty deposition.

3. *Lack of oxygen and adenosine triphosphate* (*ATP*).—The synthesis of phospholipids may be studied by measuring the incorporation of ^{32}P into the lipids of tissue slices and homogenates. Lack of oxygen and inhibition of respiration by cyanide interrupt this incorporation in tissue slices [see Chaikoff, 1942], while incorporation in particulate preparations of liver is dependent on the simultaneous oxidation of malate [Friedkin and Lehninger, 1948]. It is now known that both choline and ATP must be supplied for the synthesis of phospholipid to continue; ATP acts by taking part in the formation of cytidine-diphosphate-choline, which finally reacts with a diglyceride to produce phospholipid [see Dawson, 1957; Kennedy, 1957]. Lack of oxygen, inhibition of respiration or of oxidative phosphorylation

thus interrupt the supply of ATP, which is essential for the synthesis of phospholipids; these factors may thus induce deposition of fat.

4. *Lack of protein.*—Protein, like phospholipid, may be an essential constituent of the micelles which disperse fat. Lack of dietary protein causes deposition of fat in the liver of rats [Best, Hartcroft, Lucas and Ridout, 1955]; this deposition commences in the peripheral cells of the lobules in contrast to fatty change caused by lack of choline, which initially occurs in the central cells. Deposition due to lack of protein possibly depends on the necessity for adequate protein in the dispersion of fat within mitochondria and other micellar structures. The work of Danielli and Harvey [1935] indicates that protein exercises important effects at intracellular lipid-water interfaces. Lipoprotein complexes may thus play a part in the dispersion of fat; disintegration of such complexes or micelles through lack of protein may accordingly be responsible for the deposition of fat.

5. *Excess of cholesterol.*—The feeding of cholesterol is a potent cause of deposition of fat in various organs [see Cook and McCullagh, 1939]. As we have seen, the dispersion of fat in micellar form is promoted by phospholipid; but cholesterol antagonizes phospholipid in its action on emulsions. Cholesterol may thus cause deposition of fat by impeding the action of phospholipid in forming micelles containing dispersed fat. Cholesterol also appears to interfere with the synthesis of phospholipid [Perlman and Chaikoff, 1939]. The resultant loss of micellar fat would, moreover, interrupt utilization, so that accumulation of excess of fat also is a sequel to the action of cholesterol. The hypercholesterolæmia of diabetes may thus be responsible (see below) for fatty deposition which is so prominent a feature in this disease.

B. *Increase of Quantity of Intracellular Fat*

Excess of fat to an extent which cannot be accommodated in the dispersed micellar state may also result in the deposition of visible droplets of fat. Excess of fat in the cell can materialize in two principal ways:

1. By increased entry of fat into the cell.
2. By diminished removal of fat from the cell.

1. Increase of entry of fat into the cell might take place as a result of damage to the cell membrane. Lipæmia, however, is a more likely cause of increased entry; lipæmia arises from excessive mobilization of fat from the depôts. This may account for deposition of fat in the liver during starvation and wasting diseases. Lipæmia may contribute to fatty deposition in diabetes, although excess of cholesterol in this disease, as we have seen, may more directly convert micellar into globular fat.

2. Diminished removal of fat from the cell may eventuate in three ways depending on the three principal fates of intracellular fat. Fat in the cell (i) may be oxidized, (ii) may be phosphoryated to phospholipid, or (iii) may be mobilized from the cell in a dispersed state as fat itself. It is likely that fat must be dispersed in micellar form for each of these mechanisms of removal to operate; therefore the mere conversion of micellar to globular

fat would impede utilization irrespective of the method of disposal. Even, when fat is not entering the cell as such, inhibition of the mechanisms of removal can result in accumulation, since fat may arise *de novo* in the cell by transformation from carbohydrate.

(i) *Diminished oxidation.*—The oxidative katabolism of fatty acids takes place in two major stages (fig. 8). First each molecule of fatty acid is degraded by successive β-oxidation so as to liberate one molecule of acetyl coenzyme A (acetyl Co-A) at each succeeding step, until finally one molecule of acetoacetic acid is left as a remnant. In the second stage acetyl Co-A may have three principal subsequent fates: it may enter the tricarboxylic (Krebs cycle) and become oxidized to CO_2, it may condense to acetoacetic acid, or it may take part in the synthesis of cholesterol.

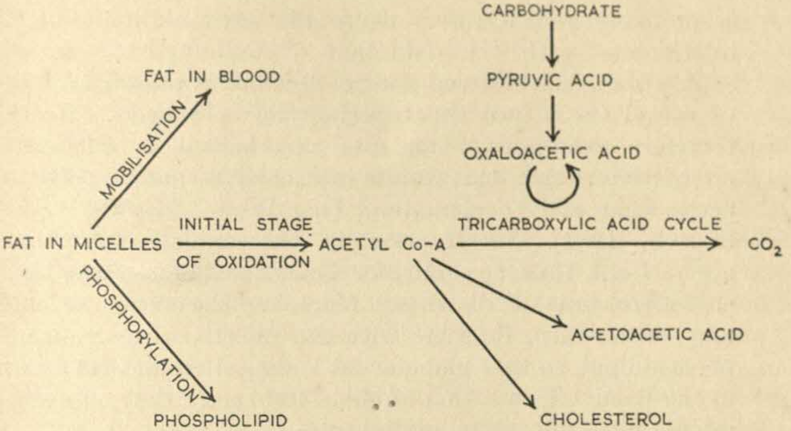

FIG. 8.—Fate of cellular fat

Interruption of the initial stage of degradation of acetyl Co-A may cause accumulation of fat by impeding its utilization. Artom [1953] showed that the formation of CO_2 and acetoacetic acid from fatty acids (labelled with ^{14}C) is defective in the fatty livers of rats on a diet deficient in choline; the supply of choline to these animals restores the capacity of the liver to oxidize fatty acid to acetoacetic acid and CO_2. Artom [1953, 1955] suggested that impairment of oxidation together with accumulation of fat is due to lack of phospholipid, which is not formed in the absence of choline. Micellar fat in the mitochondria is probably the substrate for oxidation. Dawson [1957], as we have seen, regarded phospholipid as essential for maintenance of mitochondrial integrity and considered that lack of phospholipid may cause accumulation by interfering with oxidation of fat in the mitochondria. According to the view now proposed micellar fat would in any case be replaced by globular fat in the absence of sufficient phospholipid. Thus lack of choline would, in the first place, cause deficiency in phospholipid and so directly result in the appearance of globular fat. Moreover the concomitant loss of micellar fat may produce defective oxidation along with accumulation which then leads to further deposition of visible globules.

Derangement of the subsequent katabolism of acetyl Co-A may also accompany the accumulation of fat. This later stage, however, does not directly affect the removal of fat itself and so its interruption is probably not a direct cause of accumulation. In diabetes interference with the oxidation of carbohydrate impedes the supply of oxaloacetic acid (see fig. 8) which is essential for the entry of acetyl Co-A into the tricarboxylic acid cycle. Acetyl Co-A therefore accumulates and its metabolism is deflected along alternative paths; this results in excessive condensation to acetoacetic acid and to cholesterol [see White, Handler, Smith and Stetten, 1954]. Interruption in the oxidative katabolism of acetyl Co-A thus accounts for both the ketosis and the hypercholesterolæmia of diabetes. Moreover the excess of cholesterol may, in its turn, interfere with the micellar dispersion of fat by phospholipid, so that globular fat is deposited and fat accumulates in the liver. This action of cholesterol may thus account for the intense fatty deposition of diabetes.

(ii) *Diminished removal as phospholipid.*—Fat is phosphorylated to phospholipid through the stage of a diglyceride. Choline and ATP [see Dawson, 1957; Kennedy, 1957] are essential contributors to this synthesis. Deficiency of choline, or of ATP, thus interrupts the conversion of fat to phospholipid and so would tend to cause accumulation of fat in the cell. Lack of oxygen and lack of gluscose, or indeed inhibition of oxidative phosphorylation due to any cause, can deplete the stores of ATP in the cell and so impede the synthesis of phospholipid.

If fat is normally removed from the cell by phosphorylation and transported as phospholipid, then interruption of the formation of phospholipid from fat would account for accumulation of cellular fat. There is, however, a great objection to this conception. Studies on animals deficient in choline by Zilversmit, Entenmann and Chaikoff [1948] revealed that choline does not stimulate the turnover of phospholipid in the plasma, although it does stimulate the turnover of phospholipid in the liver. These workers concluded, therefore, that choline does not act by increasing the transport of fat away from the liver in the form of phospholipid, but by stimulating the utilization of fat itself in the liver. It is thus likely that conversion to phospholipid and *removal in this form* is not a major fate of cellular fat, and that interference with *this* process of removal cannot substantially account for the accumulation of cellular fat in disease.

(iii) *Diminished mobilization as fat itself.*—Another possibility (see fig. 8) is that substantial quantities of fat are normally mobilized and removed from the cell as unaltered fat. Doubtless this mobile fat is dispersed in micellar state, since visible droplets could hardly penetrate the cell membrane with any facility. Phospholipid is essential for promoting this dispersion. This view readily explains how lack of phospholipid results in defective removal of fat. Fat, as we have seen, is not utilized by conversion to phospholipid and removal in this form; but a supply of phospholipid is essential for the mobilization of *fat itself.* Without phospholipid mobile micellar fat is replaced by immobile globules of liquid fat; mobilization becomes defective and excessive fat accumulates in the cell. Defective micellar dispersion which causes faulty mobilization may thus be one of the principal causes of accumulation.

According to this view deposition of fat due to deficiency in choline has a dual mechanism. Firstly, lack of phospholipid leads directly to the appearance of liquid globules and the loss of micellar fat. In the second place, loss of micellar fat impedes mobilization and the resultant excess of fat in the cell gives rise to further deposition.

To sum up we may say that the intracellular deposition of fat firstly arises from interference with dispersion so that visible droplets are formed; lack of phospholipid may be responsible for this change. Secondly, deposition may arise owing to accumulation of fat in excess; this may occur as a result of excessive entry into the cell or of defective utilization. Since micellar fat is probably the form which takes part both in oxidation and mobilization, deficiency in micellar fat is one of the principal causes of accumulation. Accumulation thus accompanies deposition both as an effect as well as a cause. One factor, conducive both to deficiency in micellar fat and to defective utilization, is lack of phospholipid, which is probably essential both for mobilization as well as oxidation. It is thus evident that lack of phospholipid is a potent factor in determining deposition, either directly by impeding micellar dispersion, or indirectly by impeding utilization and so causing accumulation, which in its turn gives rise to further deposition of visible fat.

CAUSES OF DEPOSITION IN DISEASE

As with other disorders of the cell deposition of fat in disease *either* may be the first consequence of injury to the cells initially damaged *or* else may arise indirectly in cells remote from the primarily injured tissue. Before considering how various mechanisms of fatty deposition may operate in cellular injury and disease we must briefly discuss this question of direct and indirect injury.

Direct Injury

Frequently the deposition of fat occurs as a direct sequel to adverse conditions or to the action of harmful substances. Thus lack of choline in the diet apparently produces fatty deposition in the cells first affected by this deficiency. The toxin of *Corynebacterium diphtheriæ* may similarly cause deposition of fat in heart muscle which is directly injured by the toxin itself.

Indirect Injury

In many cases, however, damage to one tissue or cell group indirectly affects other living elements, so that fatty deposition develops in these indirectly injured cells far from the initial injury. Moreover, the initial cause of cellular damage may give rise to cellular reactions which later injure other cells in a different manner from the original harmful mechanism. Thus the reaction of the initially damaged protoplasm may bring about changes of second, third or even higher orders in other cells whose malfunction then results in the deposition of fat.

The following example will serve to illustrate the kind of sequence of events involved in deposition of fat produced by indirect injury. Diabetes in rats, following injection of alloxan, is often accompanied by intense deposition of fat in the liver. Plate I, 1 shows the liver of a rat killed 3 days after an injection of alloxan (200 mg./kg. body weight); the hepatic cells are filled with numerous droplets of liquid fat. Plate I, 2 shows an islet of Langerhans from the pancreas of this diabetic rat, while Pl. I, 3 illustrates an islet from a normal rat, for comparison; both these sections were stained with Gomori's paraldehyde fuchsin to show the specific granules in the β-cells, which normally contain the precursor of insulin. Abundant β-cells stuffed with purple granules fill the whole centre of the islet from the normal rat (Pl. I, 3). The islet from the diabetic rat, however, is completely devoid of β-cells (Pl. I, 2). Clearly the primary injury is death and destruction of the β-cells which fabricate insulin; but the way in which lack of insulin secures deposition of fat is obscure. Possibly a servo-mechanism, involving discharge of fat from the depôts through the mediation of the pituitary gland, may cause lipæmia and so lead to excessive entry of fat into the hepatic cells. Alternatively interference with katabolism of carbohydrate may result in the accumulation of acetyl Co-A and cholesterol, which can itself cause deposition.

We thus see that fatty deposition *either* may occur in the cells first injured *or* may arise in cells far from the primary damage as an indirect consequence of the initial cellular lesion. In the following brief discussion on the cellular mechanisms, involved in causing fatty deposition in individual diseases, examples of both direct and indirect injury will be encountered.

Malnutrition

Starvation causes mobilization of fat and so indirectly stimulates the entry of fat into the liver. Chronic malnutrition may result in lack of choline and so directly produce defective synthesis of phospholipid. Deficiency in protein may, as we have seen independently interfere with the dispersion of fat and cause peripheral deposition of fat in the lobules of the liver.

Grossly enlarged fatty livers are common in malnourished children in the tropics. The supply of choline does not cure this condition, which may be due to multiple deficiencies. The livers of these undernourished children are severely depleted of protein [Waterlow, 1954].

Human Diabetes

Lack of insulin appears to be the primary defect in the type of diabetes commonly seen in younger patients [Lawrence, 1951; Bornstein and Lawrence, 1951]. Here faulty metabolism of carbohydrate with accumulation of cholesterol (fig. 9) may be responsible for fatty deposition in the liver. Cholesterol, as we have seen, may promote deposition of fat by directly antagonizing the emulsifying action of phospholipid. It is also possible that defective metabolism may indirectly stimulate discharge of fat from the depôts and so produce lipæmia. Lipæmia may be intense in diabetes [see Best, 1946] and this may cause excessive entry of fat. In the milder type of diabetes, encountered in elderly corpulent patients, lack of insulin is not the basic disorder [Bornstein and Lawrence]; in this case excessive mobilization from the depôts probably increases the entry of fat into the cells of the liver.

Diseases which cause Lack of Oxygen

Heart failure and anæmia produce deposition of fat in the liver. This change is generally most pronounced in the central or midzonal cells of the liver lobules, while the peripheral cells are frequently unaffected. Rappoport, Borowy, Lougheed and Lotto [1954] consider that the liver is composed of berry-like masses of cells; each of those structural units ensheathes the terminal branches of the entering vessels. The central regions of each of these "acini" correspond to the peripheral parts of the classical "lobules" (which are in close contact with the branches of the portal vein and hepatic artery). The outer regions of the acini similarly correspond to the central parts of the lobules and also to those peripheral and midzonal portions of the lobules which are remote from the entering vessels. This view of hepatic topography correlates well with the zonal distribution of anoxic damage. In heart failure and anæmia fatty deposition first occurs in cells situated at the periphery of the *acini*, which are most poorly supplied with oxygen. Lack of oxygen doubtless interferes with the formation of ATP and so interrupts the synthesis of phospholipid.

Sometimes the centrilobular cells of fatty livers contain less visible fat than the midzonal cells; this feature may be conspicuous following congestive heart failure. Possibly intense deposition depends in part on the formation of fat *de novo* from carbohydrate. This process is probably less effective in the centrilobular cells, which are the most poorly supplied with sugar. Once the onset of deposition is initiated by lack of oxygen, or other causes of cellular injury, the further supply of fat may be necessary to secure the most abundant deposition.

Fatty change in heart muscle tends to be most pronounced in the inner parts of the ventricular wall, where the blood-vessels are emptied of blood by compression during systole and where anoxia is therefore the most severe. In Addisonian anæmia fatty change is most conspicuous in the groups of fibres which are farthest from the arteriolar branches of the coronary arteries; thus the "thrush breast" distribution of fatty change arises in the myocardium. The zones of unaffected fibres which ensheath the entering blood vessels, resemble the similar zones in the hepatic "acini" in their insusceptibility to injury.

Poisons, Toxins and Infections

These may cause deposition of fat by inhibiting respiration. Chronic poisoning by cyanide thus produces fatty change in the liver. Inhibition of respiration may act by impeding oxidative phosphorylation. Methylene blue, thionine and Janus green, which interfere with oxidative phosphorylation, even without inhibiting respiration, also cause deposition of fat [Dianzani and Scuro, 1956]. In all these cases loss of cellular ATP may interrupt the synthesis of phospholipid, so that fatty deposition ensues as a sequel to lack of phospholipid.

Poisons, which cause fatty deposition, may at the same time produce irreversible cellular injury and death. Carbon tetrachloride causes intense fatty deposition in the liver and also kills many of the cells. In rats given carbon tetrachloride fat may appear in all but the peripheral cells of each hepatic lobule. In addition many of the central cells are rapidly killed or else undergo hydropic swelling [Cameron and Karunaratne, 1936]. Plate II, 1 illustrates the liver of a rat poisoned by carbon tetrachloride, which shows conspicuously lobular distribution of both fatty and hydropic change. The peripheral cells of each *lobule* are uninjured and no fat is visible within them. The outermost fatty cells are midzonal in position; they contain abundant droplets of fat (blackened by osmium tetroxide), but otherwise appear undamaged (Pl. II, 2). The line of demarcation between the peripheral uninjured cells and these grossly fatty midzonal cells is often extremely sharp. More centrally the cells are hydropic (Pl. II, 2). These hydropic cells are

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PLATE I, 1.—Frozen section of liver from an alloxan-diabetic rat. Intense fatty deposition. Oil red O and Carazzi's hæmatoxylin. ×580.

PLATE I, 2.—Pancreatic islet from same alloxan diabetic rat, showing complete loss of β-cells. Stained Gomori's paraldehyde fuchsin after treatment with unacidified $KMnO_4$ [see Dixon, 1955]; counterstained 1:10 Loeffler's methylene blue. ×400.

PLATE I, 3.—Pancreatic islet from a normal rat. Stained as in Pl. I, 2. Purple stained β-cells predominate. ×400.

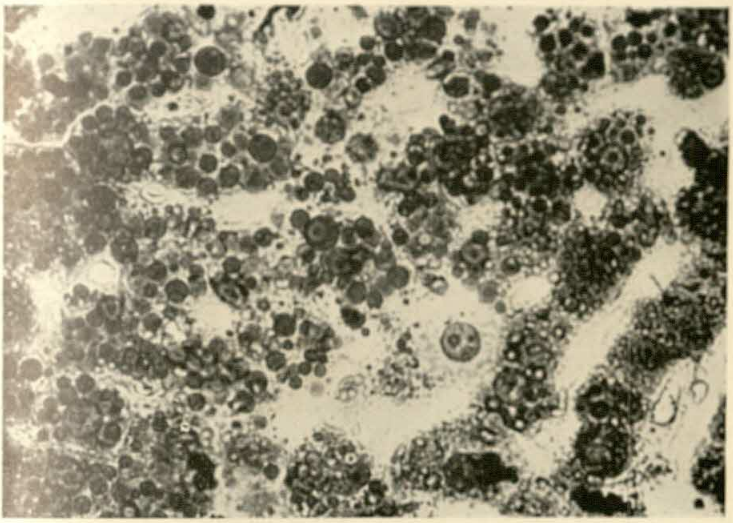

PLATE I, 1.

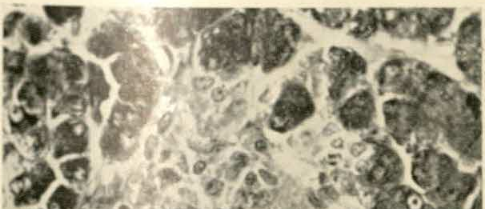

PLATE I, 2.

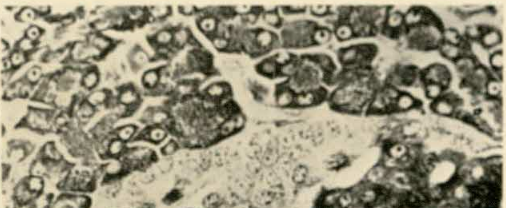

PLATE I, 3.

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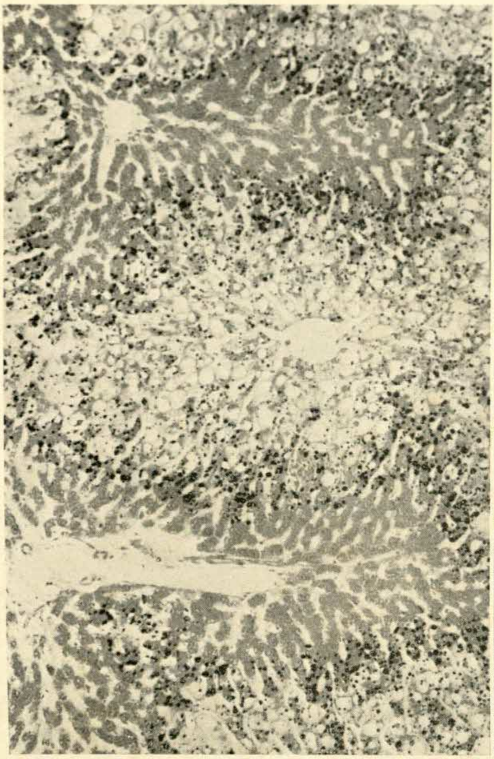

Plate II, 1.

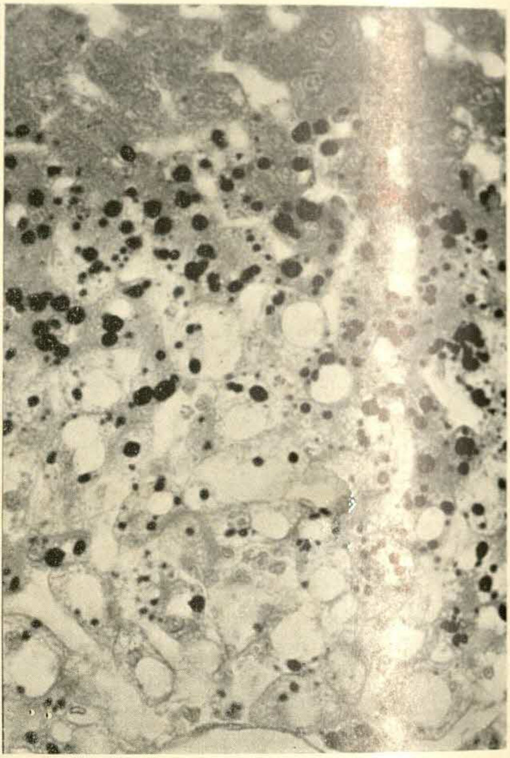

Plate II, 2.

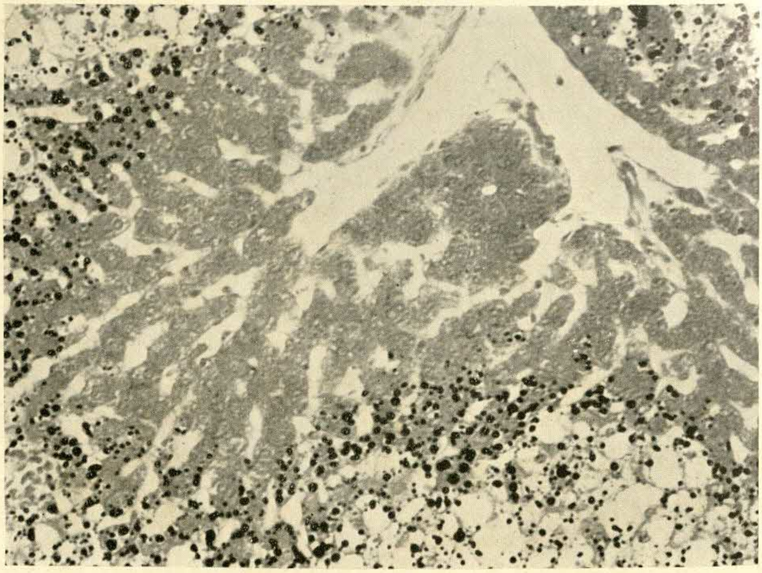

Plate II, 3.

depleted of most of their protein [Dixon and McCullagh, 1957], but contain small droplets of fat as well as large aqueous vacuoles.

The lobular distribution of hepatic lesions, which is so frequently observed after administration of carbon tetrachloride, suggests that anoxia may contribute towards injury of the cells. The peripheral cells of each lobule display a relative immunity both to cellular damage and to fatty change; this protection may depend on an adequate supply of oxygen. As we have seen, Rappoport and his colleagues regard each "acinar" mass of cells, supplied by the terminal branches of the entering blood-vessels, as the fundamental unit of hepatic structure. This view is consistent with the remarkable integrity to damage by carbon tetrachloride commonly displayed by the central cells of each of these vascular zones. Plate II, 3 shows an acinar mass of undamaged cells in immediate proximity to the terminals of an entering vessel. It is possible that good oxygenation protects the cells by ensuring a plentiful supply of phospholipid. If carbon tetrachloride injures cells by removing phospholipid or by impeding its synthesis, then injury would be most severe in those cells which are poorly supplied with oxygen and most deficient in phospholipid. The sharp midzonal transition from normal to fatty cells (Pl. II, 2 and 3) may correspond with a ratio of fat to phospholipid, which must be surpassed before visible droplets are formed. Beyond this point the concentration of phospholipid may be so diminished that fat is deposited as visible globules. Once the dispersion of micellar fat is impaired, utilization becomes defective so that fat accumulates giving rise to further secondary deposition. In the cells farthest from the entering blood vessels, where anoxia is even still more severe, the level of phospholipid may be so lowered that the cells are killed or become hydropic in addition to suffering fatty change.

Whether carbon tetrachloride gives rise to fatty deposition by dissolving phospholipid or by inhibiting respiration is uncertain. Christie and Judah [1954] found that carbon tetrachloride inhibits the respiration of mitochondria. However, the cellular respiration of slices from the fatty livers of guinea-pigs poisoned by carbon tetrachloride is not lower than in similar tissue from normal guinea-pigs [Ennor, 1942]; this indicates that carbon tetrachloride may act independently of any effect on respiration.

Harmful substances may injure mitochondrial structure at the same time as causing deposition of fat. The mitochondria are likely to be one of the

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Plate II, 1, 2 and 3 are from sections of liver of a rat fed approximately 2·5 g. CCl_4 over 48 hr. and then killed. Tissue fixed in OsO_4 and embedded in wax. Sections stained with Pyronin G.

Plate II, 1.—Lobular disposition of fatty change is conspicuous. Centrilobular cells are hydropic as well as fatty. ×100.

Plate II, 2.—Same field as in Pl. II, 1 showing transition from unaffected to fatty and then to hydropic cells. ×410.

Plate II, 3.—Illustrates integrity of cells which ensheath the entering blood vessels. The cells which are remote from the entering blood suffer fatty and hydropic change. The demarcation between normal and fatty cells is abrupt. ×190.

principal intracellular structures available for the dispersion of fat in the invisible or micellar state. When the mitochondrial dispersion disintegrates the fat becomes aggregated into liquid droplets. Christie and Judah [1954] showed that carbon tetrachloride swells mitochondria into vesicular bodies stainable by Sudan IV. Removal of phospholipid may be concerned in this effect. In mice poisoned by phosphorus the mitochondria of acinar cells of the pancreas fuse into droplets of lipid [Scott, 1916]. Moreover the α-toxin of *Clostridium welchii* disintegrates the phospholipid of isolated mitochondria [MacFarlane and Datta, 1954]; this action of the α-toxin may be related to the deposition of fat which occurs in muscle during gas gangrene [MacFarlane and MacLennan, 1945].

Fatty change may be prominent in various diseases caused by infective agents. In tuberculosis, for instance, all the cells of the liver may be bloated with fat. The cause of this change is undetermined. Diphtheria also gives rise to fatty change; this may depend on inhibition of cellular respiration by the toxin, which is itself related to the protein moiety of the iron-phorphyrin respiratory pigments [see Pappenheimer, 1955]. The ease with which harmful agents inhibit respiration, and so ultimately interfere with the synthesis of phospholipid, may often account for fatty change in disease. Deficiency of phospholipid, which appears to be a common sequel to cellular injury, is thus probably one of the principal determinants of fatty deposition in disordered cells.

> From Natures's chain whatever link you strike,
> Tenth, or ten thousandth, breaks the chain alike.
>
> Alexander Pope, *Essay on Man*, 1734.

Acknowledgments

The sections illustrated in Pl. II came from material obtained in experiments conducted in collaboration with the late Dr. G. P. McCullagh. Mr. P. J. Hart made the preparation shown in Pl. I, 1; to him and to Mr. W. A. Mowlam I am grateful for technical assistance. I also wish to thank Mr. S. W. Patman for the photomicrography and Mr. H. Mowl for making the diagrams.

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THE AMINO ACIDS AND PHOSPHOETHANOLAMINE IN SALIVARY GLAND SECRETIONS OF NORMAL MEN AND OF PATIENTS WITH ABNORMAL CALCIUM, PHOSPHORUS AND AMINO ACID METABOLISM.

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Samples of parotid gland secretion from four normal volunteers were examined for ninhydrin reacting substances by paper chromatography and electrophoresis. Samples of normal whole saliva and normal submaxillary gland secretion from a volunteer were similarly examined. Parotid gland secretion from seven patients with metabolic bone disease and with widely differing plasma alkaline phosphatase concentrations, one with cystinuria and one with polycythæmia vera were similarly examined.

The ninhydrin reacting substance present in highest concentration in normal parotid and submaxillary secretions was phosphoethanolamine. Normal whole saliva contained a considerably lower concentration of phosphoethanolamine than the two major salivary secretions. A substance was found in it which was not observed in parotid nor in submaxillary secretions. This is believed to have been γ-amino butyric acid but it could have been δ-amino valeric acid. Parotid secretion from a cystinuric showed no excess of cystine, lysine, arginine or ornithine.

Parotid secretion from a woman with hypophosphatasia showed a normal concentration of phosphoethanolamine. The concentration of phosphoethanolamine in parotid secretion was not related to the concentration of alkaline phosphatase in the plasma.

THE amino acid content of human saliva has been investigated by Kesel, O'Donnell and Kirch [1945]; Kirch, Kesel, O'Donnell and Wach [1947 and 1950]; Goldberg, Gilda and Tishkoff [1948]; Rosebeek [1949]; Berry [1951]; Berry and Cain [1951]; Tasman and Smith [1953]; Fosdick and Piez [1953]; Capozzi [1954] and Woldring [1955]. These workers have examined whole saliva, obtained by asking the subjects to spit. In view of the fact that whole saliva is a mixture of secretions, from the various salivary glands, containing micro-organisms, food particles and cell debris it was felt desirable to examine the amino acids of the individual secretions of the major salivary glands. This paper describes such an investigation in normal and abnormal subjects, in which extensive use was made of paper chromatographic and electrophoretic techniques to identify the ninhydrin reacting substances.

METHODS

Subjects.—H. B., A. K., G. P. and A. R. are normal men aged 32, 31, 30 and 31 respectively. In addition nine patients were studied. Of these seven had metabolic bone diseases, one was a cystinuric and one had polycythæmia vera. Details of their clinical conditions are given in the appendix.

Collection of Secretions.—Samples of parotid secretion were obtained using the cannulation technique described by Kerr [1955]. Submaxillary gland secretion was collected from normal A. K. For this purpose a fine polyethylene cannula (0·28 mm. bore, 0·61 mm. external diameter, 2 cm. long) fused to the end of a larger polyethylene tube (1·2 mm. bore) was inserted into Wharton's duct so that the shoulder formed by the junction of the two tubes was pressed against the opening. Individual gland secretions were collected under resting conditions or with added stimulation in the form of sweets, lemon juice or paraffin wax chewing. Whole saliva of normal H. B. was collected by spitting, with and without the addition of paraffin wax chewing. Each sample as it was produced was delivered by the collecting tube or funnel straight into a container surrounded by iced water. The rate of output of all samples was recorded.

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* Dental Research Fellow, Guy's Hospital.

The subjects were sitting down or lying in bed. Collections were not limited to any particular time of day or any particular time relationship with meals. None of the subjects showed any obvious body water deficit which might have interfered with the production of saliva [Adolph, 1947].

Particular care was taken to prevent artefacts due to bacterial contamination of the samples and possible metabolism of amino acids. Specimens of parotid and submaxillary secretion obtained by the methods described were submitted for bacterial examination and reported to be sterile. The whole saliva samples produced a rich growth of micro-organisms. In all cases secretions were chilled immediately and kept at a cool temperature until ultrafiltration which removed bacteria and enzymes. Secretions from individual glands were observed to be quite clear and colourless after delivery of the first 1–2 ml. which were rejected.

Preparation of Specimens for Analysis.—Each sample was ultrafiltered as soon as possible at 0–4° C., either immediately or on the following day. In the latter case the specimen was frozen solid during the intervening hours. The solution was passed through an ultrafiltration shell (Membranfilter Gesellschaft, Germany) using a suction pressure of 660 mm. of mercury applied to the outside of the shell. Each ultrafiltrate was desalted by the micro-modification [Dent, 1951] of the electrolytic method of Consden, Gordon and Martin [1947]. Desalted ultrafiltrates were stored at $-10°$ C.

Chromatographic Methods.—Desalted ultrafiltrates were examined for amino acids by the standard two-way paper chromatographic technique of Consden, Gordon and Martin [1944], using aqueous phenol solution in the presence of ammonia vapour as the first solvent, and a lutidine and water mixture [Robson and Rose, 1957] in the presence of diethylamine as the second solvent. In each case a 2 ml. sample was applied to the paper after preliminary evaporation to a volume of approximately 20–50 μl.

In the cases of the parotid secretions of P. S. and E. F., chromatograms were also made after oxidation with hydrogen peroxide and ammonium molybdate. Such oxidation is necessary to detect the presence of cystine and methionine [Dent, 1947].

Electrophoretic Methods.—One ml. of each desalted ultrafiltrate was examined for amino acids by the paper electrophoretic method of Harris, Mittwoch, Robson and Warren [1955], using the buffer of pH 5·5, a potential of 220 V. and a duration of 13–15 hr. Under these conditions phosphoethanolamine is well separated from other ninhydrin reacting substances. The normal parotid secretion of A. K. was also subjected to electrophoresis at pH 11·5.

Radio-phosphorus Experiment.—Patient W. C. was given a therapeutic dose of 5 mc. of ^{32}P intravenously at 2.30 P.M. on July 12th. Both parotid ducts were then cannulated and the secretions collected from both sides from 2.45 to 4.00 P.M. and the specimen ultrafiltered on that day. On the 13th a sample was desalted and subjected to paper electrophoresis. The paper strip was exposed to an ordinary X-ray plate for 94 hr. from 3.45 P.M. on the 14th until 1.45 P.M. on the 18th. Desalted ultrafiltrate was submitted to the two-way chromatography from the 13th to the 15th, and the chromatogram exposed to an X-ray plate for 68 hr. from 1.30 P.M. on the 15th until 9.30 A.M. on the 18th. The radiographs were then developed and the electrophoretic and chromatographic papers treated with ninhydrin in the usual way.

RESULTS

Chromatograms

Tracings of the spots on representative chromatograms of desalted ultrafiltrates of secretions from normal parotid and submaxillary glands are shown in figs. 1 and 2 respectively. There were slight variations from subject to subject. The result for the parotid secretion of the polycythæmic W. C. closely resembled those from the normals H. B., A. K. and G. P. Fig. 3 shows the chromatogram from the whole saliva of H. B. On these tracings the number next to each spot represents the intensity of colour using an arbitrary 0–10 scale [Dent, 1947]. The letters indicate the identities of the spots. Inspection shows that the ninhydrin reacting substance present in the highest concentration in normal parotid secretion is one which moves to the position which would be expected for phosphoethanolamine, and that there is no other ninhydrin reacting substance present in comparable concentration. The presumption that this spot was due to phosphoethanolamine was strengthened by the following tests and others described under the heading *Paper Electrophoresis*.

Phosphoethanolamine marker ran to this position when run with other markers only, and it coincided with the spot when run with parotid secretion on the same chromatogram. Both phosphoethanolamine and the spot were unaffected by oxidation with hydrogen peroxide and molybdate before chromatography. The stippled area in fig. 1 shows the area of radioactivity revealed by the autoradiograph of the chromatogram obtained from the parotid secretion of W. C. after intravenous injection of radio-phosphorus. Comparison showed that the area of radioactivity was in the same position and had the same shape as the ninhydrin reacting spot which was presumed to be phosphoethanolamine. This spot must therefore have contained phosphorus. The autoradiograph revealed only one other spot, a large one, in the position of inorganic phosphorus ion and quite distinct from the presumed phosphoethanolamine spot.

The chromatogram of submaxillary gland secretion of normal A. K. differed from that of parotid secretion in that the higher molecular weight amino acids, tyrosine, phenyl alanine, leucine and lysine were not found in the former. As in the case of parotid secretion the chromatogram of submaxillary secretion gave a spot in the phosphoethanolamine position, of strength comparable to that of the corresponding spot in the parotid secretion chromatograms. The rigorous characterization tests were not, however, applied to the submaxillary secretion.

The chromatogram of whole saliva shown in fig. 3 differs from that of parotid secretion in several respects. The spot in the phosphoethanolamine position is no stronger than the other spots. The concentrations of most of the amino acids are similar to those in the two secretions. Glycine, however, is present in considerably higher concentration in the whole saliva which also showed a new and relatively strong spot in the position of γ-amino butyric

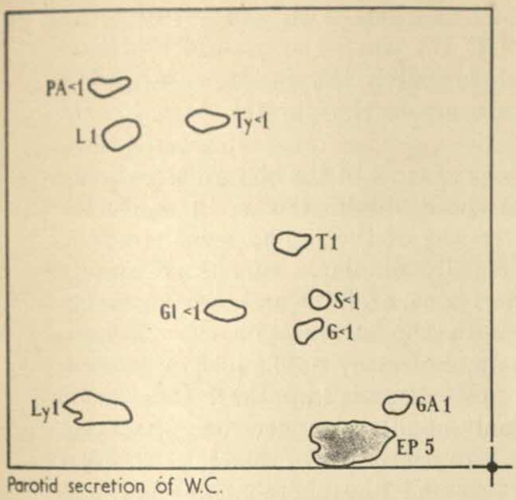

FIG. 1.

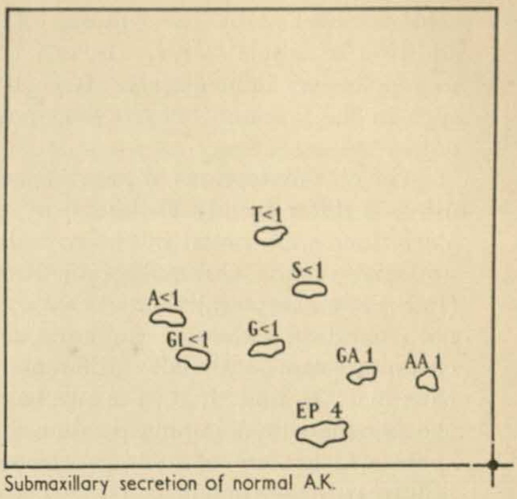

FIG. 2.

FIG. 1.—Ninhydrin reacting substances in a chromatogram of normal human parotid secretion. Here and in figs. 2 and 3, 2 ml. of prepared secretion was applied at the bottom right hand corner. Phenol was run from right to left and then lutidine was run upwards. The identifications are: A, alanine; AA, aspartic acid; AB, γ-amino butyric acid; EP, phosphoethanolamine; G, glycine; GA, glutamic acid; GL, glutamine; L, leucine; Ly, lysine; PA, phenylalanine; S, serine; T, taurine; Ty, tyrosine. The stippled area shows the area of radioactivity, revealed on the autoradiograph, which was not due to inorganic phosphate. It is very slightly smaller than the corresponding ninhydrin spot but has the same position and shape. The number next to each spot indicates the intensity of colour.

FIG. 2.—Ninhydrin reacting substances on a chromatogram of normal human submaxillary gland secretion. For explanations see fig. 1 and the text.

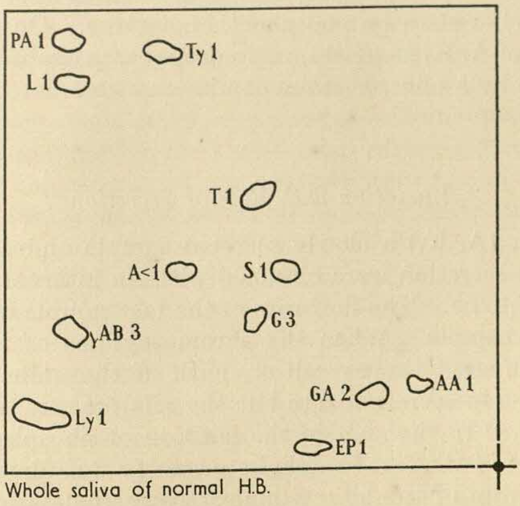

FIG. 3.—Ninhydrin reacting substances on a chromatogram of normal human whole saliva. For explanations see fig. 1 and the text.

acid as checked by the running of known markers with and without the addition of whole saliva. In one case (H. B.) when the parotid secretion was collected immediately after the whole saliva the latter contained a spot in the γ-amino butyric acid position whereas the parotid secretion did not.

The chromatograms of parotid secretions of most of the abnormal patients did not differ in any striking way from those obtained from the normals. Variations were noted in the overall strengths of the amino acids, but the patterns of the chromatograms were usually similar. In the cystinuric (P. S.) it was especially noted that cystine, lysine, arginine and ornithine were not found in excessive amounts, either in the straight or the oxidized chromatograms. Definite differences were, however, noted in some cases; thus in E. B. and M. B., the two cases of osteogenesis imperfecta the spot in the phosphoethanolamine position was only slighly stronger than the other spots which were of average strength. In the case of renal glomerular failure with osteomalacia (E. F.), the presumed phosphoethanolamine spot was weaker than most of the other spots and therefore in relatively low concentration.

Paper Electrophoresis

Analyses were carried out on parotid secretions of normals A. K., A. R. and W. C. and of patients E. G., E. B. and M. B. using a buffer of pH 5·5. In every case a spot was found in the position of phosphoethanolamine and the strength of this spot agreed with the strength of the corresponding spot on the comparable chromatogram. A radioactive spot was found in the electrophoretic strip from parotid secretion of W. C. It agreed in shape and position with the corresponding ninhydrin reacting spot on the same strip and which was believed to be phosphoethanolamine. On electrophoresis of parotid secretion of A. K. at pH 11 a strong spot was again found to be in the position taken up by a phosphoethanolamine marker.

Variation with Rate of Secretion

In one subject (A. K.) a slowly secreted sample and a quickly secreted sample of parotid secretion were obtained with an interval of only a minute between the collections. The flow rate of the fast sample was eighteen times that of the slow sample. When the chromatograms of the fast and slow samples were compared the overall strength of the amino acids decreased slightly with a rise in secretion rate but the relative strengths remained the same. With regard to the spot in the position of phosphoethanolamine, in the chromatogram of the fast sample its intensity was about half that found in the chromatogram of the slow sample. Nevertheless in all specimens of normal parotid secretion examined it was the strongest ninhydrin reacting substance present.

DISCUSSION

From the evidence given above it was concluded that the substance thought to be phosphoethanolamine was in fact this compound. Thus the strongest ninhydrin reacting substance in normal parotid secretion is phosphoethanolamine.

Comparison of the parotid secretion of normal H. B. with the whole saliva obtained from the same individual a few minutes earlier reveals some striking differences. The lesser concentration of phosphoethanolamine in whole saliva might be explained on the basis of a dilution of parotid secretion with the other secretions which go to make up the whole saliva. This is unlikely because submaxillary secretion also contains a relatively high concentration of phosphoethanolamine and between them the parotid and submaxillary glands normally contribute more than 75 per cent of the whole saliva [Schneyer and Levin, 1955]. An alternative explanation is that an enzyme is present in whole saliva but absent from submaxillary and parotid secretions, and which is capable of hydrolyzing phosphoethanolamine. In favour of this explanation is the observation of Chauncey, Lionetti, Winer and Lisanti [1954] that alkaline phosphatase activity is present in whole saliva but absent from parotid secretion. They found that acid phosphatase was present in parotid secretion, but Strickland, Thompson and Webster [1956] have shown that phosphoethanolamine is not appreciably affected by the acid phosphatases of human brain, kidney, prostate, liver and intestine. It is, however, rapidly hydrolyzed by the alkaline phosphatases of these tissues.

The occurrence of phosphoethanolamine in parotid and submaxillary secretions and in whole saliva has not as far as we are aware, been previously reported. However, Capozzi [1954] found, on paper chromatography of whole saliva, an unidentified ninhydrin reacting spot which appears in his figure to be in the position of phosphoethanolamine. Woldring [1955] found, by ion exchange chromatography, an unidentified ninhydrin reacting substance, and examination of his diagrams suggests that the unidentified peak next to taurine could have been due to phosphoethanolamine.

The concentrations of all the amino acids of parotid secretion were very much lower than the concentrations generally found in plasma [Dent, 1951]. Techniques available to us did not allow us to estimate the concentration of phosphoethanolamine in plasma. Without this information it is difficult to assess the significance of the finding of this substance in parotid (and submaxillary) secretion. It might have been expected that when the plasma alkaline phosphatase was raised or lowered, the plasma phosphoethanolamine concentration would inversely reflect the change. Similarly it might be expected that the plasma phosphoethanolamine concentration would affect the concentration of phosphoethanolamine in parotid secretion unless the phosphoethanolamine was chiefly a product of the gland cells. This argument was not, however, supported by the results obtained from patients with high and low plasma alkaline phosphatase concentrations. M. P., the woman with a plasma alkaline phosphatase concentration of 1–3 King Armstrong (K.A.)

units showed an excess of phosphoethanolamine in her urine but the concentration of this substance in her parotid secretion was normal. Of the three patients with raised plasma alkaline phosphatase the phosphoethanolamine concentration of the parotid secretion of one (E. F.) whose plasma alkaline phosphatase concentration was 72 K.A. units was low, but in the other two (L. D. and M. Y.) with plasma alkaline phosphatase concentrations of 44 K.A. units and 20 K.A. units respectively the parotid secretion phosphoethanolamine concentration was normal.

Whole saliva contained a relatively high concentration of a substance which moved on the chromatogram to the position of γ-amino butyric acid. It was not found in the submaxillary or parotid secretions. It was therefore either a product of a minor oral gland or else was a metabolite resulting from bacterial or enzymic action. Moor and Gilligan [1951] found a relatively high concentration of γ-amino butyric acid in all three specimens of whole saliva which they examined by two-way paper chromatography. They state that this amino acid together with glycine accounts for two-thirds of the total amino nitrogen of saliva. Our results are in agreement with this figure. Capozzi [1954] found that γ-amino butyric acid was the strongest ninhydrin reacting substance in whole saliva. Again we agree. In our work, however, the identity of this substance was not rigorously proven, and in view of the findings of Berry [1951] it might have been δ-amino valeric acid. This substance is stated by Fosdick and Piez [1953] to occupy the same position on the two-way paper chromatogram. Dent [1948], however, thinks the two substances occupy different positions. Clearly further work is required to elucidate this point.

It is of interest that the parotid secretion of cystinuric P. S. was indistinguishable from normal. In particular there was no excess of cystine, lysine, arginine or ornithine, the amino acids in excess in the urine of this and other cystinurics [Robson and Rose, 1957]. This is taken to mean that the amino acids pass into parotid secretion at low concentration, and that there is no mechanism of specific reabsorption of amino acids from parotid secretion comparable with the renal tubular reabsorption of amino acids. Alternatively if such a mechanism exists in the parotid ducts it is not affected in cystinuria in the way in which the renal tubular amino acid reabsorption mechanism is affected.

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We would like to thank Professor C. E. Dent and Professor M. A. Rushton for encouragement. We are also indebted to Professor Dent for allowing us to investigate his patients with metabolic bone diseases, to Dr. Gwen Hilton for allowing us to study the patient with polycythæmia, to Miss J. McAlister for technical advice on the radio-phosphorus experiment and to the volunteers for their generous co-operation.

APPENDIX

Clinical Notes on the Abnormal Subjects

W. C., male, aged 62, diagnosed as polycythæmia vera in October 1953. Since then he has been treated periodically with 5 mc. doses of ^{32}P and with satisfactory responses. For this study he was considered to be a normal man.

M. P., female, aged 40, with partial fractures of the thighs, a plasma alkaline phosphatase concentration of 1–3 K.A. units and with excess phosphoethanolamine in the urine, as described, in childhood, by McCance, Morrison and Dent [1955]; Fraser, Yendt and Christie [1955] and McCance, Fairweather, Barrett and Morrison [1956].

L. D., female, aged 41, with steatorrhœa, osteomalacia and hyperparathyroidism, who has been extensively investigated [Davies, Dent and Willcox, 1956]. The present investigation was carried out while she had steatorrhœa with "primary" hyperparathyroidism and osteomalacia, just before the removal of a second parathyroid adenoma.

E. F., female, aged 41, with chronic renal failure for seven years. At the time of this investigation she was under examination because of osteomalacia which had developed secondarily to the renal failure.

M. Y., female, aged 56, with Fanconi syndrome of the adult type. She has been described by Milne, Stanbury and Thomson [1952]. Since then her osteomalacia has recurred and at the time of our observations she was being re-investigated for this condition.

E. G., male, aged 37, with renal tubular osteomalacia, as described by Dent and Harris [1956] under the heading "Severe osteomalacia presenting in early adolescence or adult life and very closely mimicking deficiency of vitamin D". One of their patients has been described in greater detail [Rose, 1956]. At the time of this study E. G. was admitted for further investigation and was found to be recovering from an overdose of calciferol.

E. B., a girl, aged 6, with osteogenesis imperfecta. She had fragile bones, blue sclerotics, lax ligaments, flat feet and a family history of the disease.

M. B., female, aged 55, with osteogenesis imperfecta tarda, with the characteristics described under E. B. and, in addition, high tone deafness.

P. S., male, aged 37, with cystinuria. He has been described by Harris, Mittwoch, Robson and Warren [1955] and by Robson and Rose [1957].

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ABSORPTION OF RADIOACTIVE BACTERIA LABELLED WITH ^{35}S METHIONINE FROM THE APPENDIX OF THE RABBIT. By J. B. ENTICKNAP.* From the Department of Pathology, Guy's Hospital Medical School, London, S.E.1.

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Suspensions of living bacilli made radioactive by growth in the presence of ^{35}S have been injected into the lumen of the appendix in eight rabbits. Absorption of radioactive material has been demonstrated by monitoring experiments and radioautographs have shown that for periods up to 6 hr. this is due to the presence of radioactive bacteria in the mucosa and mesenteric nodes. Some incidental data on the rate of passage of intestinal contents are recorded.

INTRODUCTION

NUMEROUS bacteria are regularly present in the submucosa of the healthy rabbit's appendix [Bizzozero, 1885, and see Enticknap, 1953] and recent investigations have shown that this symbiosis has both immunological [Digby and Enticknap, 1954] and nutritional [Enticknap, 1956] significance. Furthermore, this infiltration of healthy tissues by potentially pyogenic organisms [Enticknap and Baker, 1946] is remarkable because of the complete absence of any associated pathological changes [Enticknap, 1953]. The present experiments were undertaken further to clarify the origin of these bacteria and in particular to indicate whether absorption from the bowel lumen played a part in maintaining the infiltration. Some incidental information on the uptake of methionine by the strain of gram positive bacillus studied and also on bacterial movements throughout the alimentary canal has been obtained.

MATERIAL AND METHODS

A strain of Gram positive *Bacillus* (GB 3) from the mucosa [Enticknap, 1953], was made radioactive by growth on infusion agar to which a solution of ^{35}S Methionine had been added. Several synthetic and methionine deficient media were much less effective. Three 7 ml. slopes were inoculated and the 12-hr. growth washed three times with distilled water. All washings after this showed background activity only and the supernatant fluid remained free from activity for many hours.

The tip of the appendix of eight adult field rabbits was exposed under intravenous nembutal and ether anæsthesia through 3 cm. incisions under the costal margins. Bacteria suspended in 1–4 ml. of saline were injected into the lumen through a 26 s.w.g. needle and the animals were killed with nembutal between 1·5 and 22 hr. after injection. *Monitoring* was performed on specimens of approximately equal weight collected at necropsy from the following sites:

(1) For estimation of absorption of bacteria: appendicular wall, mesenteric lymphoid mass, spleen and blood. Portions of appendicular wall were cut

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from the serosal surface to include submucosa without opening the lumen; in each animal this was subsequently confirmed by transverse histological sections of the same areas after embedding them in paraffin.

(2) For estimation of absorption of bacteria into the distal bowel: appendicular contents and fæcal material from junction of appendix and cæcum, the middle of the cæcum and the cæco-colic junction, the middle of the colon and the rectum.

(3) For estimation of retrograde passage: contents of ileo-cæcal junction, ileum, duodenum and stomach in four animals only.

The tissues were minced on to glass slides and dried over phosphorous pentoxide for 24 hr.; fæcal and blood specimens were similarly dried. Counts were made in a mica windowed E.M.H. 2 β-end counter in a castle at a constant distance of about 2 mm. The mean of 20,000 background counts was 7·563 per min. and there were no significant differences between 10 min. periods between 8.0 A.M. and 8 P.M. Two hundred background counts should therefore occur in every period of 26 min. 26 sec. Most experimental counts were made on 26 min. periods and were considered significant if they exceeded 200 by greater than twice the root mean square shown in the column $m = 200$ in the tables of Behrens [1951]. On most occasions that a significant result was obtained the background level was shown to be normal in the succeeding period. A few observations were recorded over different periods.

Autoradiographs were made by coating dried smears of active bacterial suspensions and sections from the paraffin blocks with *Kodak* stripping emulsion. There were then stored for 2–3 weeks in the refrigerator at 10° C. before developing. About 200 sections, of appendicular wall, mesenteric lymphoid mass and spleen, were examined by phase contrast illumination and some of them in greater detail after staining with methylene blue.

RESULTS

Activity of Bacterial Preparations.—Six active bacterial preparations were made on different days and smears from four of these, of smaller diameter than the window and 4 mm. from it, gave off more than six counts per million organisms per minute. There was a tendency in log-log graphs to a linear increase in radioactivity of the bacteria both with increase in the activity and the amount of methionine added to the medium.

Monitoring Experiments.—Radioactive discharges from the injected bacteria were detected in one or more sites in all eight rabbits (see Table I), but in the three to which preparations of low activity were given (R 165, R 167 and R 166) it was present in the bowel only. In the other five animals activity was detected in the mesenteric lymphoid mass and in three of these (R 163, R 168 and R 161) in the submucosa as well. Thus absorption of radioactive material by the mucosa was demonstrated as soon as 1½ hr. after injection of radioactive bacteria into the lumen and in five out of the eight animals examined. The amount of activity measured was small (see Table II) but was shown to be significant ($p < 0{\cdot}05$) with special tables calculated for evaluating counts close to mean background level [Behrens, 1951]. In the animal allowed to survive overnight (R 164) absorbed material was confined to the mesenteric nodes. Control specimens from untreated rabbits gave background activity only.

By 1½ hr. the luminal contents of the appendix had already begun to empty into the cæcum (R 163) but had not completely done so even by 6 hr. (R 166). The 22-hr. sample was the only one from the rectum that showed any activity (R 164). A remarkable retrograde passage to the duodenum occurred after 3 hr. (R 168 and R 166).

TABLE I.—ABSORPTION OF RADIOACTIVE BACTERIA INTO DIFFERENT SITES IN THE EIGHT RABBITS WHO RECEIVED INTRA-APPENDICAL INJECTIONS OF GB 3 LABELLED WITH ^{35}S METHIONINE.

Hr. after injection	1·5	2	2·5	3	3·5	4	6	22	
Activity of GB 3 suspension in counts/million/min.	46·0	1·7	16·0	1·4	6·6	6·0	1·7	46·0	
No. of rabbit	163	165	159	167	168	161	166	164	
Site examined	Result								Methods used for detecting activity
Spleen	0	0	0	0	0	0	0	0	Monitoring
Blood	0	0	0	0	0	0	0	0	Monitoring
Mesenteric lymphoid mass	+	0	+	0	+	+	0	+	Autoradiography Monitoring
Submucosa of appendix	+	0	+*	0	+	+	0	+†	Autoradiography Monitoring
Lumen of appendix	+	+	+	+	0	+	+	0	Monitoring
Appendico-cæcal junction	+	+	–	+	+	–	+	+	Monitoring
Mid-cæcum	+	0	–	+	+	–	+	+	Monitoring
Cæco-colic junction	–	0	–	0	0	–	0	0	Monitoring
Mid-colon	–	+	–	0	0	–	0	0	Monitoring
Rectum	–	0	–	0	0	–	0	+	Monitoring
Ileo-cæcal junction	–	0	–	0	+	–	+	–	Monitoring
Ileum	–	0	–	0	+	–	+	–	Monitoring
Duodenum	–	0	–	0	+	–	+	–	Monitoring

* This specimen was not monitored.
† On this specimen autoradiography gave a positive result but monitoring did not.

Autoradiographs.—Discrete opaque grains of silver halide about 0·5–1 μ in size representing individual β-discharges were seen in all preparations and only slides in which background activity accounted for less than two grains in each field at a magnification of ×1100 were examined in detail. Autoradiographs of smears of the four good preparations were visible to the naked eye as grey discs. In them the bacterial bodies were readily identified by their characteristic shape and metachromatic reaction under phase contrast illumination. The grey appearance was due to the numerous silver halide grains of which there was about one per cell, located about 1–2 μ from the originating bacterium. Removal and re-insertion of the phase plate enabled either structure to be examined in turn. Resolution by standard optical systems of methylene blue stained smears gave even clearer results but did not permit of this manœuvre. A few bacteria showed three disintegrations per cell and some others none at all. These appearances were also regularly seen in the large numbers of sections examined, both in relation to the luminal bacteria and also to those in the appendicular wall and mesenteric nodes. In several of them radioactive masses were seen close to the tips of the papillæ confirming that luminal bacteria do penetrate the *crypts*. Bacteria were usually present singly in the *epithelium* but one vacuole containing two radioactive organisms was seen. Some were also seen in the substance of the *papillæ*, mostly within macrophages, and also in the *mesenteric nodes*. None of the animals killed within 6 hr. showed any significant halide grains not accounted for by the presence of bacteria. In the one killed at 22 hr. however (R 164) some grains were grouped over the *littoral cells* of the perinodular sinuses, in which site no bacterial bodies could be seen. In one animal in which the submucosa was not monitored (R 159) absorption of bacteria was demonstrated by autoradiography, and in one other (R 164), killed at 22 hr., this method demonstrated radioactivity not associated with bacterial bodies that was not revealed by monitoring.

Table II.—The Results of a Typical Experiment. 48 × 10^9 Cells of Strain GB 3 with an Activity of 6·6 Counts per Million Organisms per Minute were injected into the Lumen of the Appendix of Female Field Rabbit R 168, 3·5 Hr. before it was killed. Mean of 2143 Background Counts on this Day was 7·23 Counts per Minute. Results Differing Significantly from Background Level are in Bold Type. Counts not made on the Standard Period of 26 Min. are placed in Brackets.

Site	No. of counts	Time in min.	No. of background counts in ensuing period	Time in min.
Spleen	218	26	. .	. .
Blood	220	26	. .	. .
Mesenteric lymphoid mass . .	**229**	26	. .	. .
	221	26	(100)	(13·2)
	223	26	. .	
	224	26	. .	
	229	26	200	26·9
	231	26	. .	. .
	231	26	. .	. .
Submucosa:				
tip of appendix . . .	**250**	26	188	26·3
middle appendix . . .	**243**	26	203	26·1
base appendix . . .	**266**	26	188	26
Lumen of appendix . . .	**(Very high Activity)**		. .	. .
Appendico-cæcal junction . .	**(905)**	5	. .	. .
Twelfth segment of cæcum . .	**(549)**	1	. .	. .
Cæco-colic junction . . .	188	26	. .	. .
Colon	198	26	. .	. .
	203	26	. .	. .
Distal rectum	199	26	. .	. .
Ileo-cæcal junction . . .	**241**	26	. .	. .
	241	26	. .	. .
Ileum	**276**	26	. .	. .
	274	26	. .	. .
Duodenum	**240**	26	. .	. .
	244	26	. .	. .

DISCUSSION

The strain of gram positive *Bacillus* used was one that had been previously recovered from the appendicular submucosa [Enticknap, 1953] and was typical of the commonest and histologically most prominent organisms in this site [Enticknap and Baker, 1946]. A gram positive rather than a gram negative bacillus was chosen because the latter are less regularly present, occur in smaller variety and are more difficult to visualize in sections. It grew readily on nutrient agar, incorporated radioactive methionine with avidity and did not readily liberate it again.

Although actual movement has not been shown the counting methods have clearly demonstrated translocation of incorporated radioactivity from appendicular lumen into the tissues. Histological examination of the tissues, combined with autoradiography shows that, for periods up to 6 hr., the activity demonstrated is due to the presence in them of the bacteria in which it was originally incorporated. Since, over this same period, whenever activity was demonstrated in the appendicular wall it was also present in the mesenteric lymphoid mass, and since no activity was found in the spleen or blood, it is clear that all the absorbed bacteria do not remain in the appendix. Some however must do since autoradiography demonstrated radioactivity there even after 22 hr. This was seen as free halide grains in the cytoplasm of appendicular sinus lining cells which are bathed in lymph from the regions where bacterial infiltration is normally most intense. As it was not associated with the presence of bacterial bodies it presumably indicates extraction of the products of bacterial lysis from this lymph, as opposed to enmeshing of whole bacteria as in the mesenteric lymph nodes.

Thus absorption of luminal bacteria has been shown to play a part in the maintenance of the bacterial infiltration of the appendicular submucosa, a mechanism suggested by the observation that a few iodophile rod-shaped cells, indistinguisable from those in the lumen, can usually be seen in sections of the epithelium of the papilla. Histological observations [Enticknap, 1953 and others unpublished] strongly suggest that the mesenchymal cells present play no part in this absorption which is therefore probably due to intrinsic bacterial motility; and indeed the bacteria normally present are motile. Unfortunately, in the present experiments, insufficient numbers of active bacteria were seen in the epithelium to cast any further light on this problem, nor was any evidence obtained as to what extent the infiltration is maintained by growth in the submucosa of this absorbed inoculum.

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INVESTIGATIONS ON THE IONIC BALANCE OF UDDER LYMPH AMONG CATTLE.

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In order to obtain a better insight into the metabolic processes of the bovine mammary gland, the composition of udder lymph and udder vein plasma has been investigated. The mean of eight samples expressed in m.equiv/l., are respectively for lymph and plasma: protein 6·9–19·7; chloride 109·4–101·1; bicarbonate 21·9–25·9; inorganic phosphorus 3·0–2·5; sodium 145·9–142·5; potassium 8·3–3·9; total calcium 3·8–4·4; ionized calcium 2·5–1·8; magnesium 2·0–1·7; sum of positive ions 160·0–152·5; sum of negative ions 141·2–149·2; the last figures have to be completed with the addition of figures for organic acids which, according to some individual tests, have proved to be much higher in lymph than in plasma.

There is a significant difference between lymph and plasma with respect to all tested ions, but especially those of protein, chlorides, potassium and ionized calcium. The significant difference between protein and chloride percentages tallies with lymph of other organs and with the Donnan equilibrium, but the difference of potassium and ionized calcium is typical of udder lymph and is probably related to the milk synthesis.

Typical too are the values of the mutual correlation co-efficients obtained between the different ions, some of them being significant for plasma, lymph and milk and others for milk and lymph only. Also the values of the Ca/P ratio in lymph, plasma and milk point to the important part played by the udder lymph in the milk synthesis.

INTRODUCTION

THE synthesis of milk in the bovine mammary gland cannot be studied by measurements of the composition of arterial and of venous blood alone. The lymph drainage from the udder must also be considered. Since the literature appeared to contain no reference to the composition of udder lymph the following investigation into the ionic balances in this field was undertaken.

METHODS

Cows (East Flemish and Friesian breed) in good lactation and with healthy udders were used. First a blood sample from the udder veins was taken, treated with heparin and immediately centrifuged, to prevent any influence of the cells on the mineral composition of the plasma. The animal was then killed and immediately a complete and deep dissection was made of the udder, as far as possible seeing that the lymph canals were not injured. Both udder strings, on either side of the median line and containing arteries, veins, lymph canals and nerves, were carefully ligated. The udder was then severed. Subsequently lymph was obtained by puncture of the swollen lymph canals and heparin was added. Lymph from the udder always presents as a pale yellow, slightly turbid fluid, which completely clears after centrifuging.

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[*] Associé du Fonds National de la Recherche Scientifique.

On samples of both lymph and plasma the following determinations have been made:

(*a*) Refractive index at 17·5° C. with the Zeissrefractometer (prism L 1).
(*b*) Conductivity at 25° C. with the aid of a Shedlovsky cell and a conductivity bridge.
(*c*) Protein content by the micro-Kjeldahl method [de Vleeschauwer and Heyndrickx, 1946].
(*d*) Chlorides by the iodometric titration method of Van Slijke and Hiller [1947].
(*e*) Bicarbonate by the method of Boone and Field [1953].
(*f*) Inorganic phosphate by the method of Lowry *et al.* [1954] using ammonium molybdate on a trichloroacetic acid filtrate.
(*g*) Sodium and potassium with the flame spectrophotometer of Lange.
(*h*) Calcium and magnesium with ethylenediaminetetraacetate according to the method of Piette and Piette [1955]. The percentage of calcium ions has been calculated from the total percentage of calcium and protein by means of the formula of Zeisler [1954].

Finally in a few tests citric acid, lactic acid and amino acids have been determined by the method of Natelson, Pincus and Lugovoy [1948]; Mendel and Goldscheider [1925] and Florkin [1937], respectively.

The results have been expressed in m.equiv/l.; following Gamble [1952] the value of 1·8 has been used for the valency of the phosphorus ion and the factor 2·43 has been applied for the proteins. The results have been evaluated statistically according to Snedecor [1950]. In order to find out whether the concentration of each ion differs significantly in lymph and plasma the "t" test has been applied.

RESULTS

Table I shows the mean values for eight samples of udder lymph and the corresponding plasma from udder veins. The detailed results are given in the appendix.

TABLE I.—MEAN COMPOSITION OF EIGHT SAMPLES OF UDDER LYMPH AND PLASMA OF THE UDDER VEINS (M.EQUIV/L.)

		Lymph	Plasma
Anions .	. Cl	109·4	101·1
	HCO_3	21·9	25·9
	PO_4	3·0	2·5
	Protein	6·9	19·7
		141·2	149·2
Cations .	. Na	145·9	142·5
	K	8·3	3·9
	Ca	3·8	4·4
	Mg	2·0	1·7
		160·0	152·5
	Ca^{++}	2·5	1·8

The chloride and inorganic phosphate contents attain significantly higher values in lymph than in plasma; opposed to this, the bicarbonate content of lymph is always below that of plasma. Sodium and magnesium contents are slightly higher, and that of potassium much higher, in lymph than in plasma. The calcium content in lymph is slightly lower than in plasma, but the percentage of calcium ions in lymph is always higher than in plasma.

The sum of the positive ions varied in the lymphs from 151·8 to 173·1 (mean 160·0 ± 6·6) m.equiv/l., whereas in plasma the variations are from 144·6 to 160·3 (mean 152·5 ± 6·4) m.equiv/l.

The sum of the negative ions when calculated from the ions given are, in both plasma and lymph, less than the sums of the positive ions; the difference in plasma being 3·3 m.equiv/l. and in lymph being 18·8 m.equiv/l. In the former case the difference is partly due to organic acids. The question arises whether the much larger difference in lymph is also to be accounted for by organic acids. Accordingly, in three tests, amino acids, citric acid and lactic acid have been determined. The mean contents were found to be 4, 1 and 8 m.equiv/l. in lymph as against 2, 0·5 and 1 in plasma. These figures show that the percentage of organic acids, especially of lactic acid, is very low in plasma and several times higher in lymph.

DISCUSSION

As there is no literature on the composition of udder lymph, we have to limit ourselves to comparing the results obtained with those of lymph of other origin. It appears from the data collected by Yoffey and Courtice [1956] that, in the lymph originating from the thoracic and main cervical ducts, the total base is slightly lower than in plasma, whereas the percentages of chloride and bicarbonate are higher. According to the same authors, these data correspond to the Gibbs-Donnan equilibrium between two phases whose concentrations of non-diffusible protein-ions differ.

TABLE II.—RESULTS (M.EQUIV/L.) OBTAINED FROM INVESTIGATION OF UNDILUTED AND OF DILUTED PLASMA, IN DIALYSIS EQUILIBRIUM

	Undiluted	Diluted		Undiluted	Diluted
Protein	18·9	5·4	Sodium	138·0	138·0
Chloride	102·6	109·1	Potassium	5·2	5·2
Bicarbonate	24·3	25·8	Calcium	4·2	3·4
Inorg. Phos.	3·8	4·1	Magnesium	2·6	2·5

When plasma was dialyzed against the same plasma diluted four times with a saline solution of about the same composition (Na 127·0, K 4·8, Ca 3·8, Mg 2·8, Cl 109·1, HCO_3 26·3 and PO_4 3·6 m.equiv/l.) the results, given in Table II, have been obtained. This shows that after dialysis the content of sodium and potassium are equal, whereas the content of calcium and magnesium are lower in the diluted plasma than in the non-diluted. The total base is lower in the diluted plasma as stated by Yoffey and Courtice [1956]. Similarly we find a higher percentage of chloride and bicarbonate in the diluted plasma.

Although in the udder lymph which we have investigated, the protein concentration is also much lower than in the plasma, our figures only partly tally with a Donnan-equilibrium as exists in the above kinds of lymph. The total bases and especially the potassium content of the udder lymph are higher than in the plasma; indeed the chloride percentage is higher in the lymph, but the bicarbonate percentage is lower.

Between the udder lymph and the plasma of udder veins there is a significant difference ($P = 0{\cdot}01$) regarding the percentage of all tested elements, but especially as regards that of protein, chloride, potassium and ionized calcium. The protein content is several times lower in udder lymph than in plasma this of course, characterizes all kinds of lymph and needs no further comment. The higher chloride percentage in this lymph is also not unusual. Characteristic however, of the udder lymph, is the higher percentage of potassium and calcium ions. Probably there is some relation between these higher percentages and milk synthesis. The average concentration in milk of potassium, total and ionized calcium is 43, 60 and 5 m.equiv/l. respectively.

Furthermore, it has seemed of importance to us to calculate the correlations existing between the different mineral elements in lymph and plasma. The linear relation and the correlation co-efficients have been calculated by the classical methods described by Snedecor [1950] and the most important results are given in Table III.

TABLE III.—RELATION BETWEEN THE CONCENTRATIONS (M.EQUIV/L.) OF THE DIFFERENT IONS IN LYMPH (L) AND PLASMA (P)

	Equation	Correl. Co-eff.		Equation	Correl. Co-eff.
L	$\mathrm{Mg} = 0{\cdot}05\,\mathrm{Na} - 5{\cdot}8$	$+0{\cdot}63$	L	$\mathrm{K} = 0{\cdot}20\,\mathrm{Na} - 20{\cdot}5$	$+0{\cdot}52$
P	$\mathrm{Mg} = 0{\cdot}04\,\mathrm{Na} - 3{\cdot}4$	$+0{\cdot}83$	P	$\mathrm{K} = -0{\cdot}02\,\mathrm{Na} + 7{\cdot}0$	$-0{\cdot}41$
L	$\mathrm{Mg} = 0{\cdot}16\,\mathrm{K} + 0{\cdot}7$	$+0{\cdot}70$	L	$\mathrm{Cl} = 0{\cdot}63\,\mathrm{Na} + 18{\cdot}1$	$+0{\cdot}59$
P	$\mathrm{Mg} = -0{\cdot}50\,\mathrm{K} + 3{\cdot}6$	$-0{\cdot}59$	P	$\mathrm{Cl} = 0{\cdot}50\,\mathrm{Na} + 30{\cdot}6$	$+0{\cdot}56$
L	$\mathrm{Cl} = -0{\cdot}95\,\mathrm{Bic} + 130{\cdot}3$	$-0{\cdot}71$	L	$\mathrm{Ca} = 0{\cdot}15\,\mathrm{K} + 2{\cdot}5$	$+0{\cdot}54$
P	$\mathrm{Cl} = -0{\cdot}83\,\mathrm{Bic} + 122{\cdot}6$	$-0{\cdot}57$	P	$\mathrm{Ca} = 0{\cdot}05\,\mathrm{K} + 4{\cdot}2$	$+0{\cdot}05$
L	$\mathrm{Phos} = 0{\cdot}06\,\mathrm{Bic} + 1{\cdot}7$	$+0{\cdot}40$	L	$\mathrm{Phos} = 0{\cdot}72\,\mathrm{Ca} + 0{\cdot}32$	$+0{\cdot}64$
P	$\mathrm{Phos} = 0{\cdot}10\,\mathrm{Bic} + 0{\cdot}1$	$+0{\cdot}72$	P	$\mathrm{Phos} = 0{\cdot}50\,\mathrm{Ca} + 0{\cdot}33$	$+0{\cdot}30$
			L	$\mathrm{Cl} = 7{\cdot}00\,\mathrm{Mg} + 95{\cdot}1$	$+0{\cdot}56$
			P	$\mathrm{Cl} = 5{\cdot}02\,\mathrm{Mg} + 92{\cdot}7$	$+0{\cdot}25$

There is a significant correlation of minimum $0{\cdot}70$ ($P = 0{\cdot}05$) between the following elements:

magnesium and sodium in plasma and slightly less in lymph,
magnesium and potassium in lymph and slightly less in plasma,
bicarbonate and chloride in lymph and slightly less in plasma,
bicarbonate and phosphorus in plasma.

Besides, there are the following correlation co-efficients with significance at P = 0·2 (r minimum 0·51):

sodium and potassium in lymph,
sodium and chlorides in lymph and plasma,
total calcium and potassium in lymph,
total calcium and inorganic phosphorus in lymph,
magnesium and chlorides in lymph.

Some of these correlations, such as these between sodium and chlorides are valid both for lymph and for plasma and even, at least according to the very scanty available literature, for milk. Others apply only to lymph; the total calcium inorganic phosphorus correlation is also of significance for milk. In the literature [de Vuyst and Van Belle, 1957] this last correlation is better known as Ca/P ratio which amounts in milk from 1·18 to 1·37 [Ellenberger, 1950; Black, 1934 and Elser, 1934]. This ratio averages 1·27 ± 0·18 in lymph and 1·82 ± 0·42 in plasma with a very significant "t" value of 5·50 (appendix).

So, certain typical ratios and correlations point to the close relationship between udder lymph and milk synthesis. Our further research into the matter will undoubtedly provide more data.

APPENDIX

RESULTS (M.EQUIV/L.) OBTAINED FROM INVESTIGATION OF UDDER LYMPH (L) AND PLASMA OF THE UDDER VEINS (P)

Sample	Refractive index		Conductivity		Proteins		Chloride	
	L	P	L	P	L	P	L	P
1	1·33854	1·35073	0·01369	0·01152	4·2	19·6	103·4	94·1
2	1·33873	1·35054	0·01348	0·01137	4·5	18·9	106·6	98·1
3	1·33885	1·35091	0·01369	0·01152	4·7	19·5	112·2	102·8
4	1·34241	1·35165	0·01380	0·01167	9·0	20·1	114·3	103·7
5	1·33892	1·35169	0·01403	0·01167	4·8	20·4	111·6	102·8
6	1·34260	1·35040	0·01348	0·01191	9·2	18·8	117·9	111·6
7	1·34290	1·35021	0·01242	0·01167	9·5	18·5	105·3	101·1
8	1·34264	1·35320	0·01288	0·01094	9·2	21·6	104·0	94·8
Mean	1·34070	1·35117	0·01343	0·01153	6·9	19·7	109·4	101·1
S.D.	0·00208	0·00099	0·00053	0·00029	2·5	1·0	5·3	5·6
"t" value	14·03		10·56		13·76		11·53	

Sample	Bicarbonate		Inorg. Phos.		Sodium		Potassium		Tot. Calc.	
	L	P	L	P	L	P	L	P	L	P
1	24·0	31·0	3·2	3·0	145·0	140·0	7·7	3·6	3·7	4·7
2	18·5	19·9	2·1	1·7	142·0	137·0	6·9	4·0	3·2	4·0
3	22·2	25·3	2·5	1·8	150·0	150·0	7·7	3·6	3·4	4·5
4	19·1	26·8	3·3	2·7	154·0	150·0	12·5	4·2	4·0	4·3
5	23·5	27·5	2·5	2·3	150·0	148·0	6·5	3·6	3·2	3·8
6	16·0	21·5	3·2	2·4	144·0	144·0	9·0	4·0	4·8	4·8
7	23·0	24·8	3·6	2·9	143·0	136·0	8·5	4·5	4·0	4·4
8	28·7	30·3	3·8	3·2	139·0	135·0	7·4	4·0	3·8	4·3
Mean	21·9	25·9	3·0	2·5	145·9	142·5	8·3	3·9	3·8	4·4
S.D.	3·9	3·9	0·6	0·6	5·0	6·3	1·9	0·3	0·5	0·3
"t" value	4·55		6·25		3·82		7·00		4·62	

Appendix—(*continued*)

Sample	Magnesium		Tot. Cations		Tot. Anions		Ion. Calc.		Tot. Ca./Phos.	
	L	P	L	P	L	P	L	P	L	P
1	2·1	1·8	158·5	150·1	134·8	147·7	2·8	1·9	1·16	1·57
2	1·6	1·5	153·7	146·5	131·7	138·6	2·4	1·6	1·52	2·35
3	2·6	2·2	163·7	160·3	141·6	149·4	2·5	1·8	1·36	2·50
4	2·6	1·8	173·1	160·3	145·7	153·3	2·4	1·7	1·21	1·59
5	1·6	1·8	161·3	157·2	142·4	153·0	2·4	1·5	1·28	1·65
6	2·3	1·6	160·1	154·4	146·3	154·3	2·9	2·0	1·50	2·00
7	2·0	1·5	157·5	146·4	141·4	147·3	2·4	1·9	1·11	1·52
8	1·6	1·3	151·8	144·6	145·7	149·9	2·3	1·6	1·00	1·34
Mean	2·0	1·7	160·0	152·5	141·2	149·2	2·5	1·8	1·27	1·82
S.D.	0·4	0·3	6·6	6·4	5·3	5·0	0·2	0·2	0·18	0·42
"t" value	3·27		6·52		..		14·00		5·50	

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THE UPTAKE AND METABOLISM OF ^{14}C LABELLED CHYLOMICRON FAT BY THE ISOLATED PERFUSED LIVER OF THE RAT.

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When chylomicra labelled with ^{14}C tripalmitin were perfused through the isolated rat liver, they were taken up by the liver in the absence of heparin clearing factor. When 1 ml. of labelled chyle containing 43·6 mg. of total fatty acid was added to the perfusate, about 54 per cent was taken up in 3 hr. The pre-incubation of the chyle with heparin clearing factor increased the rate of uptake to 76 per cent in 3 hr. The addition of Triton WR 1339 did not reduce the amount of fat taken up by the liver.

The chylomicron fat was rapidly oxidized to CO_2. About 7 per cent of the fat taken up by the liver was oxidized in 3 hr. in control experiments, about 16 per cent in experiments with clearing factor and 10 per cent in experiments with Triton.

Labelled tripalmitin when injected into the blood stream of rats in the form of homologous chyle, is removed from the circulation rapidly and taken up by the tissues and readily oxidized to CO_2 [French and Morris, 1957; 1958]. A large proportion of the injected fat is recovered in the liver, but it is unknown how the fat, which is present in the blood as chylomicra, enters the hepatic cells.

There is indirect evidence that some chylomicra can pass through the endothelial lining of the hepatic sinusoids [Morris and Courtice, 1956] but it is not known to what extent chylomicra can be transferred directly to the liver cells. Some chylomicron fat may be taken up by the tissues after it has been hydrolized in the circulation by a process analogous to the heparin clearing reaction [Robinson, Jeffries and French, 1954]. The rate of uptake of chylomicron fat by the tissues has been shown to be increased by the injection of heparin intravenously and reduced by inhibitors of the clearing reaction [French and Morris, 1958].

The isolated perfused liver has been used to study the problem of the form in which chyle fat is removed from the circulation by this organ. Experiments have been carried out to estimate the rate of uptake and oxidation of chylomicron fat labelled with ^{14}C tripalmitin and the effect of the heparin clearing reaction on these processes.

METHODS

Liver Perfusion.—Livers for perfusion were taken from non-starved albino rats of either sex, weighing about 250 g. The rats were anæsthetized with ether and the liver exposed through a median incision. The bile duct was first isolated and catheterized with polyethylene tubing (0·6 mm. external diameter). The portal vein was dissected free for a distance of about 2 cm. from the hilus of the liver and tributary vessels entering the vein in this region were ligated. The inferior vena cava was tied cranial to the right renal vein, and the portal vein then cannulated with a plastic catheter ("Portex" 2 mm. external diameter). The thorax was opened immediately and a plastic catheter was inserted into the inferior vena cava towards the hepatic veins. The liver with the diaphragm attached was then dissected from the animal and placed diaphragmatic surface downwards in the glass organ dish of the perfusion apparatus. The perfusion was commenced through the portal vein catheter about 5 min. after ligation of the portal vein. The venous outflow was returned to the perfusate reservoir through the catheter in the inferior vena cava. The perfusion pressure was adjusted to 18 cm. of water and maintained within 1–2 cm. of this level during the experiment.

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The perfusion apparatus was adapted from that described by Miller, Bly, Watson and Bale [1951]. The perfusion fluid, modified from the formula of Brauer, Pessotti and Pizzolato [1951] had the following composition:

50 ml. rat blood—obtained by cannulating the aorta in rats weighing about 300 g. and bleeding into vessels containing powdered heparin (Pularin-Evans Medical Supplies). The blood was filtered through gauze to remove any small fibrin clots.
50 ml. of a solution containing: 2 g. crystalline bovine albumin (Armour), 37·5 mg. sodium bicarbonate, 460 mg. sodium chloride, 21 mg. potassium chloride, 6 mg. calcium chloride, 250 mg. glucose with streptomycin and penicillin.
4 ml. 0·1 M. disodium hydrogen phosphate.
1 ml. 0·1 M. potassium dihydrogen phosphate.

The hæmatocrit value of the perfusate varied from 21 to 25 per cent. The pH was 7·4.

^{14}C labelled chyle was obtained from a rat with a thoracic duct fistula after feeding with glyceryl tri(palmitate-1-^{14}C) dissolved in olive oil [French and Morris, 1957]. One ml. of this chyle, which contained 43·6 mg. of total fatty acids, was added to the perfusion mixture. The concentration of chyle fat in the perfusion fluid was about 0·4 mg. of fatty acid per ml. The perfusate and added chyle were mixed and equilibrated at 38° C. in the apparatus before starting the perfusion.

In one group of experiments 400 mg. of Triton WR 1339 (Rohm & Haas Co., Philadelphia), was added to the perfusion fluid to give a final concentration of about 4 mg. per ml. This concentration is known to inhibit the clearing reaction *in vitro* [Robinson *et al.*, 1954]. In another group, the effect of hydrolysis of the chylomicron fat was investigated as follows: 50 ml. of blood, containing heparin activated clearing factor, were obtained by bleeding rats 2 min. after the injection of 200 units of heparin intravenously. This blood was added to 50 ml. of the electrolyte and protein solution and 5 ml. of phosphate buffer. The labelled chyle and the perfusion fluid were then incubated at 38° C. for 1 hr. before the liver perfusion was started.

Throughout the experiments oxygen was passed into the perfusate reservoir through a dispersion tower containing 0·9 per cent sodium chloride solution and then to a multi-bulbed aeration chamber. From this chamber the gas was drawn through an absorption tower containing 50 ml. of normal carbonate free sodium hydroxide solution. The CO_2 produced by the liver was absorbed in this solution which was changed at half-hourly intervals throughout the experiment. The CO_2 output of the liver was measured from these samples by precipitation as barium carbonate [Morris, 1958]. Unavoidable loss of CO_2 occurred from the surface of the liver and the CO_2 estimates thus represent minimum figures.

Sampling Procedure and Radioactivity Measurements.—The radioactivity of the chyle was measured (1) by direct plating, (2) by plating an ethanol-ether extract of the chyle, and (3) by oxidation of the chyle to CO_2 and plating as barium carbonate

[Morris, 1958]. The ^{14}C content of the perfusate was followed by taking 0·1 ml. samples from the perfusate reservoir initially and at intervals throughout the experiment and measuring the radioactivity by direct plating [French and Morris, 1957]. A thin mica end-window GM. tube was used for counting samples. All activities were corrected to zero mass.

Extraction of Total Lipid from the Liver.—At the end of each experiment, the liver was disconnected from the apparatus and perfused with 50 ml. of 0·9 per cent sodium chloride solution at 38° C. The liver was weighed, finely ground up with sand and extracted with 400 ml. of boiling 3 : 1 ethanol-ether mixture. After filtration the residue was re-extracted with ethanol-ether and again filtered. The combined filtrates were reduced to a volume of 50 ml. and the radioactivity in 0·2 ml. portions of this extract were measured by direct plating.

Total Fatty Acid Analysis.—The total esterified fatty acid content in the liver extracts and chyle samples were measured by the method of Stern and Shapiro [1953].

Measurement of Clearing Activity.—Samples of perfusate were spun at 2000 × g. for 15 min. to remove red cells. Portions measuring 0·8 ml. of the supernatant fluid were then mixed with 0·2 ml. of borate buffer (pH 7·5) and 28 μl. of fatty rat chyle. The change in optical density of these samples was measured during incubation at 37° C. for 30 min.

RESULTS

State of the Perfused Liver

Rate of Perfusion.—The flow through the livers reached a maximum during the first 1–2 hr. of perfusion and then remained constant up to 5 hr. Maximum flow varied between 8 and 12 ml./min. In preliminary experiments perfusions continued for 8 hr. but for the uptake and metabolism studies the perfusions lasted for 3 hr.

Bile Production.—The flow of bile varied between 0·2 and 0·5 ml./hr. and was usually maximum during the second hour.

Histology.—In sections stained with hæmatoxylin and eosin most of the hepatic cells appeared normal after 3 hr. perfusion. The sinusoids were often dilated and there was distension of the portal tracts which suggested œdema. In some experiments, particularly when Triton was present in the perfusate, petechial hæmorrhages appeared near the edges of the liver lobes. Normal phagocytic activity of the Kupffer cells was demonstrated when India ink was added to the perfusate. Phagocytosis of a few red cells by the Kupffer cells was seen.

The Production and Destruction of Clearing Factor by the Perfused Liver.

To see if clearing factor might be produced by perfusion of fluid containing heparin, samples of perfusate were taken at intervals throughout the 3 hr. and tested with added chyle. No clearing activity was found.

The rate of destruction of activity in perfusates containing clearing factor was also followed. No change occurred in the clearing activity of samples taken during the first 30 min. but subsequent samples showed a decrease. After 3-hr. perfusion at 38° C. about 33 per cent of the initial activity remained.

The Uptake of Chylomicron Fat by the Perfused Liver.

Three groups of perfusions were carried out: (1) controls; (2) perfusions containing clearing factor; (3) perfusions containing Triton. In each the disappearance of radioactivity from the perfusate approximated to an exponential function on time. The regression of $\log_{10}$ of the percentage of the initial radioactivity remaining in the perfusate on time was calculated from the results of each experiment. The liver uptake curves constructed from the above regressions are shown for each perfusion experiment in fig. 1.

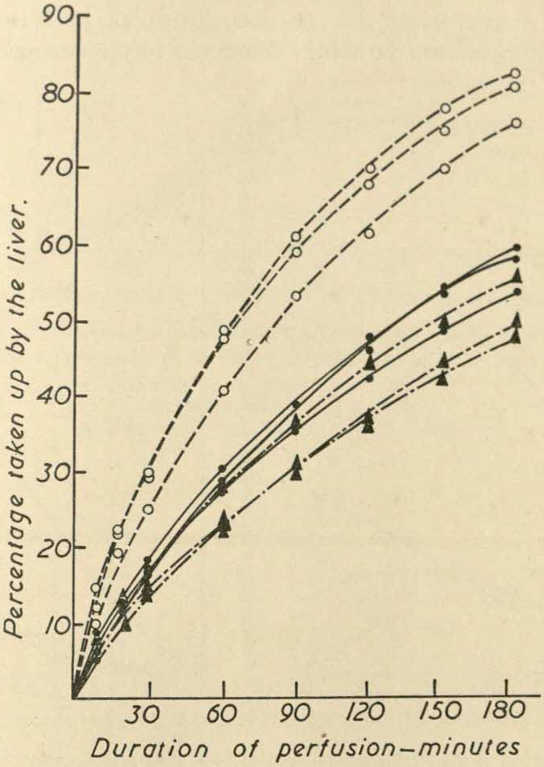

FIG. 1.—The uptake of ^{14}C labelled chylomicron fat by the perfused rat liver.

● Control experiments.
○ Experiments with clearing factor.
▲ Experiments with Triton WR 1339.

In the control experiments 54–60 per cent of the labelled chyle was taken up by the liver in 3 hr. This represented an uptake of about 25 mg. of total fatty acids.

The uptake of labelled chyle fat by the perfused liver was increased when the chyle was pre-incubated in perfusate containing clearing factor before carrying out the perfusion. This procedure removed the turbidity produced by the chyle. Seventy-five to eighty-three per cent of the chyle, representing about 35 mg. of total fatty acids, was taken up in 3 hr. (fig. 1).

In experiments in which Triton was added to the perfusate the liver took up 45–55 per cent of the chyle in 3 hr. (fig. 1) *i.e.* about 24 mg. of total fatty acids. The rate of disappearance of the labelled chyle from the perfusate was not significantly different from that in the control group.

The Oxidation of ^{14}C Labelled Chylomicron Fat by the Perfused Liver.

The percentage of the added chyle which was oxidized to CO_2 during the 3-hr. perfusion is shown in fig. 2. In each group of perfusions labelled CO_2 was given off during the first 30 min. When the chyle was pre-incubated with clearing factor the rate of conversion of the labelled fat to CO_2 was faster than in the control or Triton perfusions. At the end of 3 hr. a mean of about 8 per cent of the ^{14}C of the added chyle had been recovered as CO_2 in the experiments using clearing factor, 3·1 per cent in the Triton experiments and 2·5 per cent in the controls.

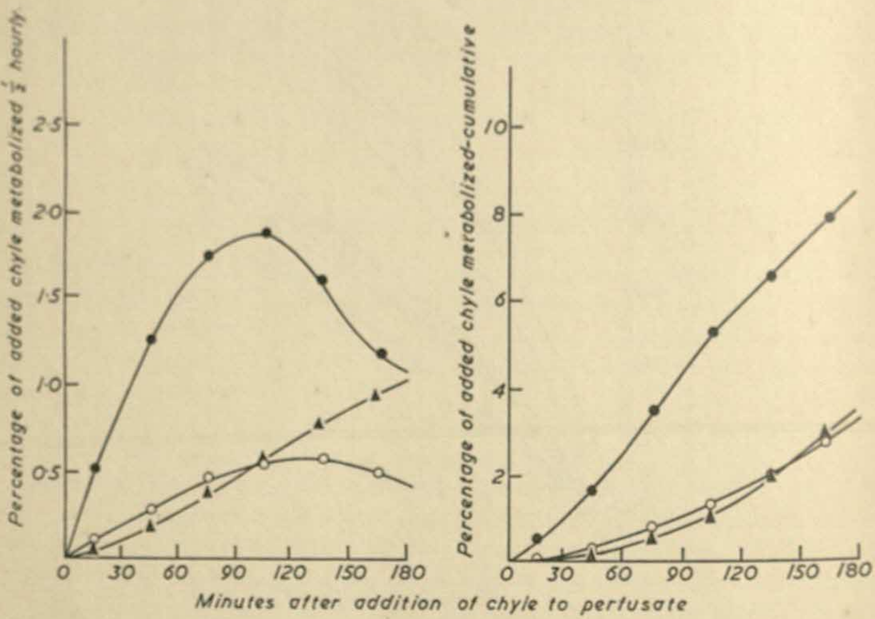

FIG. 2.—The oxidation of ^{14}C labelled chylomicron fat by the perfused rat liver. Mean results are shown for three experiments in each group.

○ Control experiments.
● Experiments with clearing factor.
▲ Experiments with Triton WR 1339.

Comparisons of the specific activity of the CO_2 given off by the livers showed that a much greater proportion of the total CO_2 production was due

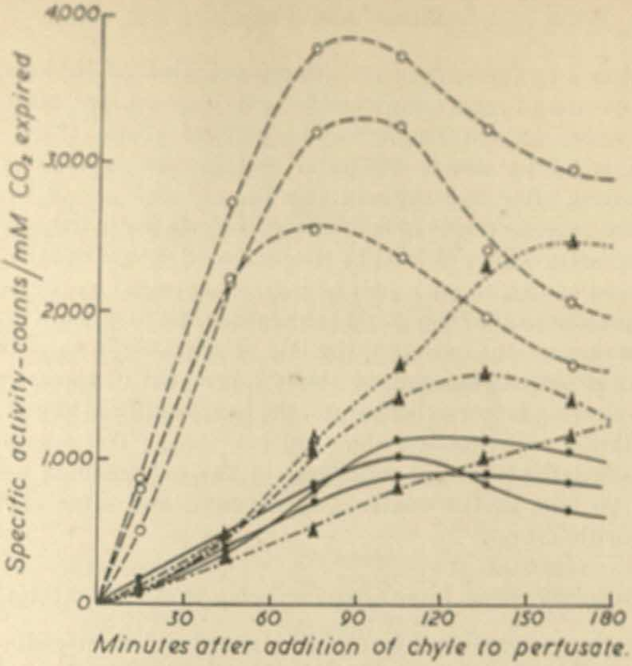

FIG. 3.—The specific activity of the CO_2 given off by the liver during perfusion with added ^{14}C labelled chylomicron fat.

● Control experiments.
○ Experiments with clearing factor.
▲ Experiments with Triton WR 1339.

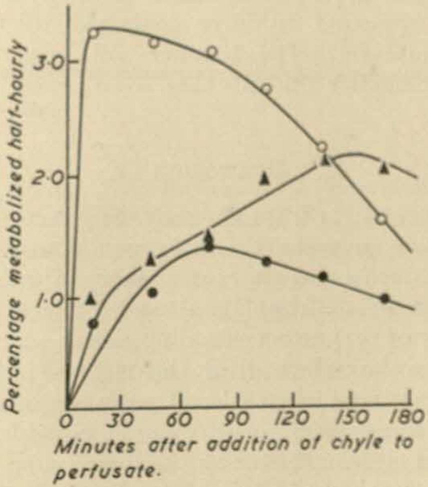

FIG. 4.—The oxidation of ^{14}C labelled chylomicron fat by the perfused rat liver. The mean results for three experiments in each group are expressed as the percentage of the fat present in the liver which was oxidized during each time interval.

● Control experiments.
○ Experiments with clearing factor.
▲ Experiments with Triton WR 1339.

to the oxidation of chylomicron fat after incubation of chyle with clearing factor. The period of maximum specific activity occurred after 60–90 min. perfusion in these experiments. In the control group the peak specific activity occurred after about 120 min. and in the experiments in which Triton was added, after 150–180 min. (fig. 3).

To see if the increased rate of conversion of chyle fat to CO_2 in experiments using clearing factor was explained by the increased rate of uptake, the amount of ^{14}C recovered as CO_2 in each half hour was expressed as the percentage of the amount present in the liver during the same time interval (*i.e.* the amount taken up less the amount oxidized) (fig. 4). Expressed in this way the livers in the control experiments oxidized about 7 per cent of the fat taken up in 3 hr.; in the clearing factor experiments, the livers oxidized about 16 per cent; and in the Triton experiments, about 10 per cent. The maximum rate of oxidation occurred in the first half hour in the experiments using clearing factor, after 90 min. in the control experiments, and after 2½–3 hr. in the experiments with Triton.

Histology of the Liver after Perfusion with Added Chyle

Frozen sections stained with Sudan IV showed a characteristic distribution of fat after amounts of chyle up to 5 ml. had been added to the perfusate. The fat was concentrated in parenchymal cells at the periphery of the lobules, and less was contained in the cells nearest the central vein. The fat was present as discrete, finely dispersed droplets which were usually localized at the margin of the cells nearest the hepatic sinusoids. In many cells near the portal tracts the droplets appeared to have coalesced to form large globules which filled the cytoplasm. The Kupffer cells also contained some fat droplets but in much smaller amounts than were present in the parenchymal cells.

DISCUSSION

The failure of the rat liver to produce clearing factor when perfused with fluid containing heparin suggests that this organ is not concerned in the production of clearing factor *in vivo* after intravenous injection of heparin. This confirms the findings of Jeffries [1954] and Spitzer and Spitzer [1956]. Although the activity of perfusate containing clearing factor decreased during the period of perfusion through the liver, the decrease in 3 hr. was of the same order as that which occurred in samples of perfusate incubated at 38° C. in a water bath. Under these conditions it was not possible to demonstrate any specific destruction of clearing factor by the liver.

The Uptake and Oxidation of Chylomicron Fat.—From results obtained in rats which had been injected with labelled chyle intravenously it appeared that the liver played an important part in the removal of chylomicron fat from the circulation [French and Morris, 1958].

The present experiments have shown that the isolated liver can take up chylomicron fat readily from the perfusate. Considerable amounts were taken up when the perfusate contained no demonstrable clearing activity. Further, when Triton was added to the perfusate, there was little effect upon fat uptake, although the concentration of Triton used was sufficient to inhibit any lipolysis by clearing factor. It can therefore be concluded that an intravascular clearing reaction is not an essential step in the uptake of chylomicron fat by the liver.

The way in which the liver may take up fat as chylomicra is, however, not known. There is no evidence that the Kupffer cells have an essential part in the process; it appears that fat particles in the form of homologous chylomicra, in contrast to the particles in artificial emulsions, are taken up only to a very small extent by the cells of the reticulo-endothelial system [Murray and Freeman, 1951; French and Morris, to be published].

TABLE I.—BALANCE CHART SHOWING THE DISTRIBUTION OF ^{14}C ACTIVITY AT THE END OF EACH PERFUSION EXPERIMENT

Experiment		Percentage remaining in perfusate	Percentage recovered from ethanol-ether extract of liver	Percentage recovered as CO_2	Percentage unaccounted for
Controls .	1	47·0	47·4	2·5	3·1
	2	50·0	40·3	2·4	7·3
	3	40·0	54·5	2·5	3·0
Clearing factor	1	22·0	62·0	6·7	9·3
	2	28·0	56·7	10·1	5·2
	3	21·0	65·5	7·3	6·2
Triton .	1	62·0	27·5	2·3	8·2
	2	40·0	46·8	3·6	9·6
	3	43·0	48·3	3·3	5·4

It has also been shown that the perfused liver will take up hydrolyzed fat very rapidly. When the triglyceride of the chylomicra was hydrolyzed by clearing factor before reaching the liver the rate of uptake was significantly accelerated. A similar increase in the rate of uptake of labelled chyle by the liver has been reported following the intravenous injection of heparin in the whole animal [French and Morris, 1958]. It appears therefore that liver cells can take up fat from the bloodstream either as unesterified fatty acid or as particulate triglyceride.

The ^{14}C that was removed from the perfusate was not all recovered in the lipid extracts of the liver and in the CO_2 which was collected. The balance chart for the experiments (Table I) shows that between 3·0 and 9·6 per cent of the total label was unaccounted for. Unmeasured losses of CO_2 from the liver surface have been referred to already [*cf*. Rostorfer, Edwards and Murlin, 1943]. In addition, conversion of labelled triglyceride to metabolic intermediaries which were not estimated in the lipid extracts may have occurred. Small amounts of ^{14}C also appeared in the bile. The amount of fat metabolized would thus be greater than is indicated by the measurements of the recovered CO_2.

The results show that when fat is hydrolyzed by clearing factor before it reaches the liver, there is an increase in the rate of its oxidation to CO_2. This increase is not necessarily due to any specific effect of clearing factor on fat metabolism in the liver cells. It seems more probable that when fat enters the cells as fatty acid rather than as triglyceride it can be oxidized more quickly since the initial step of hydrolysis has already occurred.

The presence of Triton in the perfusate did not reduce the amount of labelled fat taken up by the liver or the amount oxidized to CO_2. These results are in contrast to the findings previously reported in the whole animal [French and Morris, 1958] and are so far unexplained.

ACKNOWLEDGMENTS

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MEASUREMENT OF BLOOD FLOW AND HEAT PRODUCTION IN THE SPLANCHNIC REGION OF THE ANÆSTHETIZED SHEEP.

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Portal blood flow, total hepatic venous outflow and cardiac output were measured by the thermodilution method in sheep anæsthetized with chloralose-urethane. Portal blood flow was closely related to cardiac output and to flow in the thoracic vena cava, the correlation co-efficients being 0·87 and 0·86 respectively and the portal flow being 30·8 per cent (S.D. ± 5·6 per cent) of the cardiac output and 50·6 per cent (S.D. ± 7·0 per cent) of the thoracic caval flow. Blood flow in the portal vein prior to laparotomy was predicted from the relation between cardiac output and portal vein flow. This averaged 45·8 ml./min./kg. (S.D. ± 8·4 ml.). There was a high co-efficient of correlation (0·73) between the hepatic venous return and cardiac output. Provisional figures were obtained for heat production by the total splanchnic area by the liver and by the area drained by the portal vein.

This paper is concerned primarily with the application of the thermodilution method [Fegler, 1954, 1957 *a* and *b*] to the measurement of portal blood flow in the anæsthetized sheep. The ultimate aim is to devise a method which in conjunction with catheterization of the portal vein [Lewis, Hill and Annison, 1957] will be applicable to the conscious animal and so allow frequent measurements of portal blood flow. It will then be possible to obtain quantitative data on the absorption of metabolites from the gastro-intestinal tract.

The data to be described include measurements not only of portal blood flow but of cardiac output and flow in some of the other vessels. Since, in addition, the method also provides data for the temperature of the blood flowing to and from the splanchnic area it has been possible to arrive at some provisional figures for heat production in this area.

Materials and Methods

Fourteen Clun Forest and two Welsh Mountain sheep, 3–24 months old and 19–41 kg. in weight were used. Most of these animals were deprived of food for 24 hr. prior to experiment although a few were brought off pasture directly to the laboratory. They were anæsthetized with an intravenous injection of chloralose-urethane (3–5 ml./kg. body weight of 1 per cent chloralose in 10 per cent urethane solution). Additional anæsthetic was given during the experiment as required. Heparin (Liquemin Roche) was given intravenously in an initial dose of 800 i.u./kg. body weight and was supplemented with doses of 300 i.u./kg. at three hourly intervals.

After tracheotomy and cannulation of the right carotid artery for recording blood pressure, constantan-copper thermocouples (gauge 36), enclosed in cardiac catheters or polyethylene tubes (external diameter 1·2–2·0 mm.), were placed in the thoracic aorta by way of the right femoral artery and in the vena cava by way of the right jugular vein. By the aid of X-ray screening the tip of the latter catheter was placed 2–4 cm. posterior to the right auricle.

A thermocouple-catheter was then introduced through the right femoral vein into the vena cava 3–4 cm. cranial to the renal veins. This was done by reference to surface markings since the thickness of the abdomen precluded screening with our apparatus. The position of this thermocouple was checked when the abdomen was opened and if found to be incorrectly placed the results of the initial part of the experiment concerning flow in this vein were discarded.

Catheters for the injection of Ringer solution were introduced directly through the right jugular vein into the anterior vena cava and through the right femoral vein into the caudal part of the posterior vena cava. Cardiac output and flow in the vena cava were determined by "instantaneous" injections of Ringer solution (18–21° C.) into either the anterior vena cava or the caudal vena cava (4–10 ml.).

Total Hepatic Outflow.—In two experiments the total venous outflow from the liver was measured by recording thermodilution curves from the thoracic vena cava after blocking, by means of a balloon, the blood flow caudal to the hepatic veins [Fegler, 1957 *b*]. Injections of Ringer solution were made cranially to the block. In other experiments it was possible to ascertain total hepatic outflow by the difference between the flow in the abdominal vena cava anterior to the renal veins, and the flow in the posterior thoracic vena cava [Fegler, 1957 *a* and *b*]. The hepatic flow measured by each of these methods included blood derived from diaphragmatic and adrenal veins.

Portal Vein Flow.—The abdomen was opened by a right paracostal incision and the portal vein exposed by retraction of the viscera. A thermocouple enclosed in a cardiac catheter or a polyethylene tube was then introduced into the portal vein either by way of one of the small mesenteric veins or directly through the wall of the portal vein. In the latter procedure a purse-string suture secured the catheter so that its tip, which contained the thermocouple, rested at the porta hepatis. A catheter for injection of Ringer solution was introduced into another small mesenteric vein and tied in position with its tip in the main mesenteric vein. Thermodilution curves were obtained from the thermocouple in the portal vein by the injection of 2–3 ml. of cool Ringer solution through the catheter.

In three experiments a thermocouple—catheter in the portal vein carried a small rubber balloon which, when inflated, completely occluded the lumen of the portal vein. Thermodilution curves recorded from the posterior thoracic vena cava before and after blocking the portal vein in this way then provided by difference an approximation of the portal flow for comparison with figures derived from thermodilution curves from the portal vein itself.

In a further experiment a thermocouple-catheter with an inflatable balloon was introduced into the portal vein and placed so that the balloon rested at the porta hepatis. A short wide-bore "side" cannula was placed on the portal vein up-stream to the balloon. By inflating the balloon for short periods the whole of the portal blood flow could be diverted through the cannula, collected and measured. Thermodilution curves were obtained at intervals for comparison with the results obtained by direct measurement.

Heat Production in the Splanchnic Area.—In the experiments in which total venous outflow from the liver was determined, heat production by the whole splanchnic area, including the liver, was calculated from the flows and differences in temperature between the hepatic veins and aorta. Since thermocouples were not placed directly in the hepatic veins, the temperature of the hepatic venous blood was derived from the difference in temperature between the blood in the thoracic and abdominal vena cava. Heat production by the liver alone was given by the difference between the total heat production of the splanchnic area and the heat production of the organs drained by the portal vein.

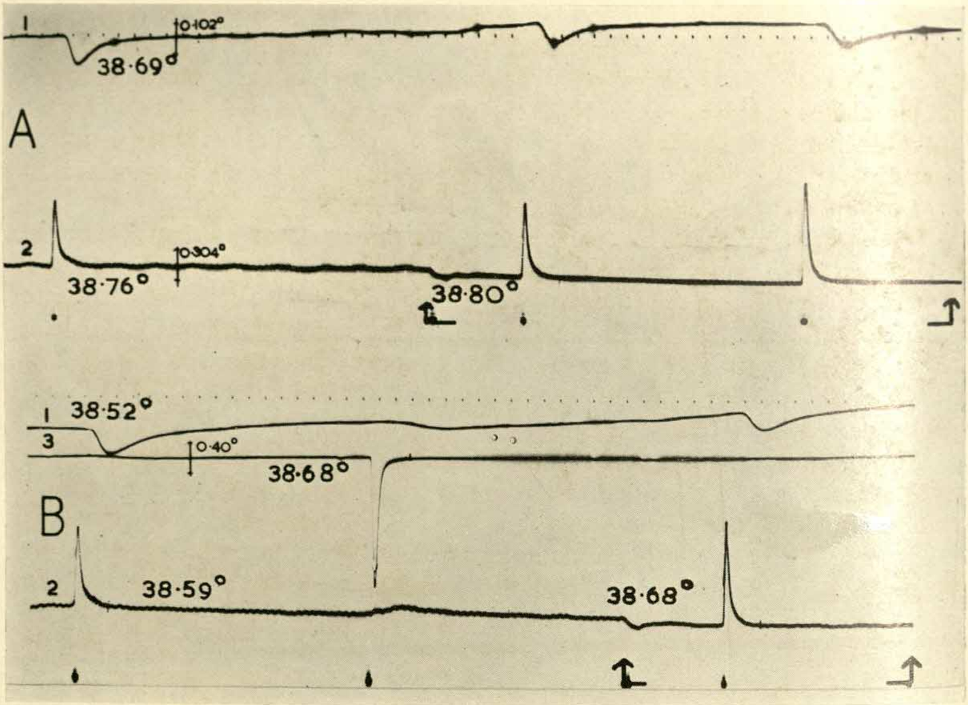

FIG. 1.—Experiment No. 1/57. Sheep ♂, 33·2 kg.

Before (A) and after (B) laparotomy. Temperature recordings (1) in aorta, (2) thoracic posterior vena cava and (3) portal vein. Time intervals = 5 sec. Single signals: in A, injections of 4, 3 and 3 ml. Ringer solution (20·5° C.) into caudal abdominal cava; in B: injection of 4 ml. Ringer solution (20·5° C.) into caudal abdominal vena cava, 3 ml. into mesenteric vein and 3 ml. into caudal abdominal vena cava. Between the arrows: blocking of abdominal cava, caudal to the hepatic veins with injections made cranially to the block. Flows calculated; A: aorta (cardiac output) 4·90 l./min.; hepatic venous outflow, 2·34 l./min.; B: aorta, 3·41 l./min., portal *v*. 1·03 l./min., hepatic venous outflow, 1·84 l./min.

Recording Instruments and Calculation of Results.—Critically damped Tinsley galvanometers (1-sec. period, sensitivity: 0·015–0·032° C./1 mm. deflection at 1 metre distance) or Cambridge Instrument Company galvanometers (1·8-sec. period, sensitivity: 0·010–0·012° C./1 mm. deflection) were used for temperature recording. Blood flow was calculated as previously described [Fegler, 1954, 1957 *a*.]

RESULTS

Typical recordings are illustrated in fig. 1 and the results of four representative experiments are given in Table I. In each of them cardiac output and blood flow in the posterior thoracic cava were determined before and after laparotomy so that the influence of surgical trauma on these values could be determined. Total venous outflow from the liver was also determined in three of these experiments.

The values for flow in the portal vein determined either by thermodilution in the vein itself or by thermodilution in the thoracic vena cava before and after blocking the portal vein were in good agreement with each other despite a marked fall in blood pressure when the portal vein was occluded [Table I, Expt. 2/55). Good agreement was also observed between the portal flow determined simultaneously in one experiment by thermodilution (0·73 l./min.) and by direct collection from the portal vein (0·77 l./min.).

The sheep seems particularly sensitive to abdominal trauma since frequently there was a considerable decrease in arterial blood pressure and in cardiac output following manipulations in the peritoneal cavity. It appeared likely therefore that the portal vein blood flow before laparotomy would be greater than that observed under the conditions of our experiments. In order to obtain some idea of what this value would be the portal vein blood flows observed after laparotomy were correlated with the simultaneously observed values of cardiac output, blood flow in the thoracic vena cava and the arterial blood pressure. The correlation coefficients for portal vein blood flow with these values were respectively 0·87, 0·86 and 0·54, indicating that the portal flow was, in fact, better correlated with cardiac output and with blood flow in the thoracic cava than with arterial blood pressure. This relation is shown graphically in fig. 2 where the portal flows measured in all the experiments are plotted against the cardiac output, and the arterial blood pressure determined simultaneously. The high degree of correlation between portal blood flow and cardiac output and between portal flow and flow in the thoracic cava suggested that it would be useful to express the mean value of flow in the portal vein as a percentage of either of these. Because it is easier to estimate cardiac output than blood flow in the thoracic cava, it was considered more practical to express portal flow as a percentage of the cardiac output. This was found to be 30·8 per cent (S.D. ±5.6 per cent). If this value, derived from laparotomized animals can be legitimately used as a basis for similar calculations on animals prior to laparotomy, then the portal vein blood flow in the latter would have been 45·8 ml./min./kg. (S.D. ±8·4 ml./min./kg.).

TABLE. I.— RESULTS OF FOUR REPRESENTATIVE EXPERIMENTS

Expt. No., sex and body weight		2/55, ♀, 28·0 kg.					9/55, ♂, 33·5 kg.			4/56, ♀, 41·0 kg.		1/57, ♂, 32·2 kg.			
Time in min. after abdominal operation		Before	117	118	120	138	Before	110	131	Before	93	Before	75	100	120
Mean arterial blood pressure mm. Hg.		132	62	*40*	*36*	55	108	52	46	110	50	137	129	115	59
Cardiac output	l./min.	4·59	2·75	*1·43*	*1·54*	2·95	4·78	2·44	2·03	4·6	2·48	4·9	3·41	3·00	2·07
	l./min./sq. m.	4·10	. .	. .	. .	. .	3·79	. .	. .	3·23	. .	3·92	. .	. .	. .
Posterior thoracic cava flow	l./min.	2·74	1·73	*0·84*	*0·64*	2·06	3·33	1·81	1·53	3·06	1·71	3·17	2·67	2·64	1·54
	per cent C.O.	59·6	62·0	. .	. .	70·0	69·5	74·4	75·0	66·1	70·0	65·0	78·0	88·0	74·4
Abdominal cava flow	l./min.	. .	. .	. .	. .	. .	0·92	0·43	0·49	1·05	0·38	. .	. .	. .	. .
	per cent C.O.	. .	. .	. .	. .	. .	19·2	17·6	24·0	22·8	15·3	. .	. .	. .	. .
Calculated total hepatic venous outflow	l./min.	. .	. .	. .	. .	. .	2·41	1·38	1·04	2·01	1·33	2·34	1·84	1·83	1·21
	per cent C.O.	. .	. .	. .	. .	. .	50·0	56·4	51·0	43·7	54·0	47·7	54·0	61·0	58·0
Portal vein flow	l./min.	. .	0·94	*0·89**	*1·09**	0·92	. .	1·01	0·85	. .	0·82	. .	1·03	1·10	0·67
	calculated flow ml./min./kg.	50·0	. .	. .	. .	. .	43·9	. .	. .	34·6	. .	45·5	. .	. .	. .
Remarks—C.O. = Cardiac output		* Portal vein blocked and reduction in post thoracic vena cava flow assumed to equal portal vein flow during this period					Hepatic venous outflow = post thoracic vena cava flow less abdominal vena cava flow.					Hepatic venous outflow = post thoracic vena cava flow when abdominal cava blocked caudal to hepatic veins.			

The two methods described for the determination of total venous outflow from the liver gave comparable results and as with the portal flow there was a high coefficient of correlation (0·73) between the hepatic venous flow and cardiac output. The mean value expressed as a percentage of cardiac output was 47·0 ± 9·4 per cent and providing the cardiac output was available it was thus possible to predict approximately total liver venous flow in those experiments where this was not determined directly. When this was done the mean initial hepatic venous outflow was 62·9 ± 7·4 ml./min./kg.

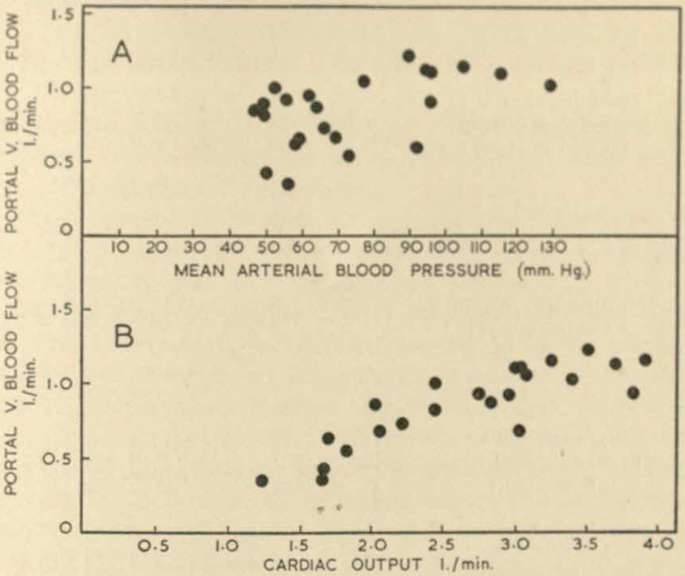

FIG. 2.—Portal *v.* blood flow plotted against art. blood pressure (A), and against cardiac output (B).

Heat Production in the Splanchnic Area

The results of experiments on dogs in which heat production in the splanchnic area was calculated from determinations of flow and temperature by thermocouples have been reported previously [Fegler, 1957 *b*]. The results of some similar determinations on the anæsthetized sheep are given in Table II. It will be seen that the mean heat production by the splanchnic area amounted to 9·9 ± 1·9 g. cal./min./kg. or about 300 cal./min./total body weight. Assuming as a very rough approximation that the total body heat production per square metre was 700 g. cal./min. then the contribution of the splanchnic area would amount to about 40 per cent of the total. There appears to be no significant difference in the contribution of the splanchnic area whether this is related to surface area or body weight.

TABLE II.—HEAT PRODUCTION BY SPLANCHNIC AREA

No. of Expt. Sex Weight kg.	Time after abdominal operation in min.	Rectal temperature °C.	Mean arterial blood pressure mm. Hg	Cardiac output l./min.	Total hepatic venous outflow l./min.	Portal vein flow l./min.	Difference in temperature hepatic vein-aorta °C.	Difference in temperature portal vein-aorta °C.	Total splanchnic area heat g. cal./min./kg.	Heat of organs drained by portal vein g. cal./min./kg.	Liver heat g. cal./min./kg.
1/55, ♂, 20·0	80	41·0	64	2·76	..	0·86	..	0·085	..	3·4	..
2/55, ♀, 28·0	120	40·7	62	2·75	..	1·02	..	0·161	..	5·4	..
3/55, ♂, 30·0	..	40·7	126	4·94	2·04	..	0·167	..	10·3	..	..
4/55, ♂, 19·2	..	39·1	96	3·08	1·14	..	0·229	..	12·4	..	..
	76	38·0	67	2·25	..	0·77	..	0·077	..	3·0	..
6/55, ♂, 40·0	80	38·5	105	3·27	1·40	1·18	0·381	0·130	12·2	3·5	8·7
7/55, ♂, 25·9	..	39·4	108	4·23	1·55	..	0·165	..	9·1	..	..
8/55, ♂, 33·3	..	38·1	79	4·89	3·09	..	0·157	..	12·9	..	..
9/55, ♂, 33·5	110	38·0	52	2·44	1·38	1·01	0·259	0·074	9·7	2·0	7·7
1/56, ♂, 35·5	..	38·0	91	4·01	2·25	..	0·121	..	7·0	..	..
2/56, ♂, 21·7	60	38·2	92	2·58	1·22	0·51	0·151	0·187	7·7	4·1	3·6
3/56, ♂, 41·5	60	38·4	96	4·00	1·34	1·17	0·239	0·020	7·0	0·95	6·1
4/56, ♀, 41·0	..	37·9	102	4·60	2·01	..	0·208	..	9·3	..	..
	93	37·8	51	2·48	1·35	0·82	0·333	0·104	9·9	1·9	8·0
1/57, ♂, 33·2	..	38·9	125	3·98	2·34	..	0·170	..	10·9	..	..
2/57, ♂, 34·8	..	39·1	108	5·14	2·02	..	0·165	..	10·1	..	..
Mean	..	..	..	..	..	..	..	..	9·9, S.D. ± 1·9	3·0, S.D. ± 1·4	6·8, S.D. ± 2·05

DISCUSSION

The only available data on blood flow in the portal vein of the sheep are those reported by Schambye [1955]. His methods (dye-dilution and infusion of ^{32}P labelled red cells) were, in principle, comparable to the thermodilution method used in the present experiments and were used by him on both conscious and anæsthetized sheep. He reports an average portal blood flow for the anæsthetized sheep of 29 ml./min./kg. with individual flows varying from 11·0 to 49·0 ml./min./kg. These figures were obtained after the abdomen had been opened and, as Schambye himself recognized, after an appreciable reduction in cardiac output had occurred.

The average figure, from the results described here, of portal flow determined after opening the abdomen was 32·6 ml./min./kg. and although this agrees reasonably well with Schambye's results both these figures are much less than the mean initial portal flow predicted from the cardiac output determined prior to laparotomy. This averaged 45·8 ± 8·4 ml./min./kg. and is somewhat greater than the 37 ml./min./kg. reported by Schambye for the conscious sheep. The close relation between cardiac output and portal blood flow observed in the present experiments emphasizes the necessity for frequent, preferably simultaneous, determinations of cardiac output when any measurement of blood flow is being attempted in the anæsthetized animal and particularly if any comparison is intended between different methods of measuring blood flow. Whether this close correlation holds in the conscious animal is not known, but experiments are now in progress in which this problem is being investigated. Should there, in fact, prove to be a close correlation between cardiac output and portal flow in the conscious animal then it will be possible to derive a good approximation of the portal flow merely by determining the cardiac output. This is of considerable practical significance since measurement of cardiac output is a relatively easy procedure compared with estimation of portal blood flow.

Although there was a fair measure of agreement between the total liver outflow measured by different methods the results were less consistent than those for portal blood flow. It seems probable however that this was mainly because of difficulties in correctly positioning the thermocouple or thermocouple-balloon in the abdominal vena cava. In the sheep there is only a short distance between the junction of the renal veins and the vena cava and the opening of the most posterior hepatic veins into the vena cava and since there appears to be considerable individual variation in this region it is difficult, by surface markings alone, to place a thermocouple or thermocouple-balloon exactly cranial to the renal veins and caudal to the hepatic veins.

On the few occasions when the thermocouple-balloon technique was used, the results were reasonably consistent indicating that a combination of occlusion of the abdominal cava and thermodilution offers a reliable technique for the estimation of total venous outflow from the liver.

The results of the experiments on heat production in the splanchnic area are in substantial agreement with similar estimations made on the dog

[Fegler, 1957 *b*]. They should, however, only be regarded as approximate for the time being since a number of factors which might influence these results have not been fully investigated. Thus allowance has not been made for the blood derived from extra-splanchnic sources, *e.g.* from the diaphragmatic and adrenal veins; nor for the possible occurrence of direct heat exchange between arteries and veins in the abdomen, particularly of the mesenteric branches [Bazett, Love, Newton, Eisenberg, Day and Forster, 1949]. It is quite possible too that arteriovenous anastomoses in the mesenteric circulation may introduce apparent anomalies in heat production by the splanchnic area. Moreover, it may be that the act of opening the abdomen, even if it is carefully closed before any records are taken, might create temperature gradients which would not appear in the intact animal. Experiments are now in progress in which these and other factors are being investigated.

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THE EFFECTS OF ACUTE HYPOXIA ON THE OSMOTIC PRESSURE OF THE PLASMA.*

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The effect of 20–60 min. periods of acute, severe hypoxia on the osmotic pressure of plasma and on the concentration of several plasma components was observed in thirty-four experiments on adult rabbits, sheep and goats, lambs and fetal sheep and goats. Changes in the pH, O_2 and CO_2 contents of the blood and in the plasma concentrations of glucose, sodium, potassium, proteins and urea were measured.

The osmotic pressure of plasma invariably rose as a consequence of acute, severe hypoxia, from 10 to 45 mOs/kg. H_2O above the normal level. In well-fed rabbits 50–100 per cent of the osmotic pressure rise was due to hyperglycemia. In starved rabbits and in the other animals 30–100 per cent of the osmotic pressure rise was due to a rise in concentration of the plasma electrolytes. In one lamb the rise in electrolyte concentration was associated with hypoglycemia. These findings are interpreted as suggesting that severe, acute hypoxia causes in every case a rise of the intracellular osmotic pressure, as a manifestation of a shift from ærobic to anærobic metabolism. The intracellular rise in osmotic pressure would result in a movement of water out of the extracellular fluids and increase their electrolyte concentration. A concomitant rise of the glucose concentration in the extracellular compartment would prevent such a shift of water.

Introduction

During the course of an investigation of the relationship between the osmotic pressures of maternal and fetal plasmas of sheep and goats, we observed that the osmotic pressure of the fetal plasma rose markedly after the umbilical vessels were occluded, provided the fetus was not permitted—or unable—to breathe [Meschia, Battaglia and Barron, 1957]. The regular association of the rise in the osmotic pressure of the plasma and the development of fetal anoxia suggested a causal relation between them and the study reported here was carried out to determine: (*a*) if acute, severe hypoxia in an animal consistently results in a rise in the total osmotic pressure of the plasma; and (*b*) the changes in the concentrations of the plasma components that are responsible for the observed elevation. The results obtained provide but a partial answer to the second of these questions inasmuch as the concentration changes in the individual plasma components followed do not, in some cases, account for the entire rise in the plasma osmotic pressure.

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‡ Josiah Macy, Jr. Fellow.

MATERIALS AND METHODS

Our experiments were carried out on fetal sheep and goats, new born lambs, adult sheep and adult rabbits. The rabbits and lambs were anesthetized with intravenous nembutal—(about 30 mg./kg. of body weight), the sheep and the mothers of the fetuses with about 30 mg./kg. of body weight of intravenous chloralose. All animals were heparinized. The fetuses were delivered by caesarean section and the first blood sample drawn promptly thereafter, from one of the umbilical vessels. The vessels were then ligated and a second sample was drawn when the heart had ceased—or was about to cease—beating. As the fetuses did not breathe, all of them being too young to initiate breathing on their own, the ligation of the cord marked the onset of hypoxia.

Our method for producing hypoxia in the lambs and full grown animals varied as our study progressed. In our first experiments, designed to ascertain if continued hypoxia would produce a rise in the osmotic pressure of the plasma, the animal breathed through a tracheal cannula into and out of a rubber bag filled with nitrogen. When respiration stopped—or appeared to be on the verge of doing so—the lungs were ventilated by gentle massage of the chest and the animal allowed to breathe air for about 10–30 sec. before being reconnected with the nitrogen bag. In later experiments the rabbit or lamb was connected through a one-way valve system to a spirometer containing a mixture of approximately 3 per cent O_2 and 97 per cent N_2; this arrangement tended to standardize the development of hypoxia and allowed the elimination of CO_2. Hypoxia in adult sheep was induced by connecting the animal to a Sanborn Basal Metabolism Apparatus, with the spirometer filled with air. So arranged the sheep breathed a mixture increasingly poorer in oxygen and the expired CO_2 was continually absorbed. When all the oxygen in the system was consumed, additional air was drawn into the spirometer.

As one purpose of our study was a determination of the changes in concentration of the plasma components responsible for the observed elevation in its osmotic pressure, the changes in concentration and osmotic pressure have been expressed in the same units *i.e.*, milliosmols (mOs) per kilogram of water (mM of glucose per kg. H_2O = mOs of glucose per kg. H_2O). The concentrations of sodium and potassium in milliequivalents/kg. H_2O have been multiplied by 0·92—their assumed osmotic coefficient—to obtain their concentrations in mOs/kg. H_2O.

On the assumption that every change in concentration of osmotically active anions, concomitant with concentration changes in plasma sodium, potassium and hydrogen ions is one of monovalent anions such as HCO_3^-, Cl^- and lactate, the resultant change in anionic concentration (ΔA^-) can be calculated from the changes in concentration of sodium (ΔNa^+), potassium (ΔK^+) and base binding capacity of the proteins (ΔP^-) thus:

$$\Delta A^- = \Delta Na^+ + \Delta K^+ - \Delta P^-.$$

Using the values of Van Slyke *et al.* [1928] relating base binding capacity of plasma proteins with pH, the approximate value of P^- in milliequivalents/liter, when the plasma protein in grams per cent (P) and the pH are known, is given by the equation:

$$P^- = 1{\cdot}1 \times P \times (pH - 5{\cdot}0).$$

Thus the change in total osmotic pressure ($\Delta\pi$) of the plasma can be related to the concentration changes of its specific components:

$$\Delta\pi = \Delta\ \text{Glucose} + \Delta Na^+ + \Delta K^+ + \Delta A^- + \Delta X$$

when Δ glucose is the change in glucose concentration, ΔX the unassigned portion of the osmotic pressure rise and all concentrations are expressed in mOs/kg. of water.

The osmotic pressure of the plasma was estimated from the freezing point depression as determined on 2 ml. samples by means of a Fiske osmometer that gives results in terms of mOs/kg. of water reliable to within ±0·5 mOs. Details about standardization of the osmometer and handling of the plasma samples have been given in a previous paper [Meschia and Barron, 1956]. (One mOs/kg. water produces a freezing point depression of 0·001858° C.) The plasma pH was measured by means of a glass electrode and a Beckman meter, reading ±0·01.

The plasma sugar concentration was determined colorometrically following the treatment with anthrone as described by Prokhovnik *et al.* [1953].* The protein concentration of the plasma was calculated from the measurement of its refractive index. The error in this case was ±0·2 g./100 ml. of plasma. A flame photometer (Baird Associates) with lithium as an internal standard was used for the estimation of the plasma concentrations of sodium and potassium. (Error ±0·5 per cent.) The micro-diffusion method of Conway as modified by Steinitz [1939] was followed in the determination of the plasma urea and the oxygen and carbon dioxide contents of the blood were determined on 1 ml. samples by means of the Van Slyke-Neill apparatus.

RESULTS

Thirty-four experiments were carried out—four on sheep and goat fetuses, two on new-born lambs, two on sheep, twenty-four on normal rabbits and two on rabbits from whom food had been withheld for 48 hr. The results obtained demonstrate (1) that there was in all cases a regular and marked rise in the osmotic pressure when the quantity of oxygen available was reduced acutely to low levels,† (2) that the elevated osmotic pressure of the plasma will return to normal value again if, after a period of hypoxia the animal is permitted to breathe room air, and (3) that the rise in the osmotic pressure of the plasma may be the result of a variety of changes in the levels of those plasma components whose concentrations we have estimated.

The rise during hypoxia and fall on return to breathing room air of the plasma osmotic pressure are illustrated graphically in fig. 1. The variations in the concentrations of the plasma components that are associated with the elevation in the osmotic pressure may be illustrated by the following cases:

In experiment 9/22/56 (Table I) on a rabbit the total osmotic pressure of the plasma rose 21·3 mOs, from 298·2 to 319·5 in the 40 min. period of hypoxia; at the same time its glucose concentration rose 24·9 mOs and the milliosmolal concentration of the ions fell 3·6. That is to say the entire rise in plasma osmotic pressure can be attributed to the elevation in plasma glucose. When the animal was permitted to breathe room air following hypoxia, the total osmotic pressure of the plasma fell 6·7 mOs in the first 60 min. In the same

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* The plasmas of sheep and goat fetuses contain fructose as well as dextrose. Both substances react with anthrone; if the reaction is carried out at 100° C. for 7 min. a color is obtained with fructose that is about 8 per cent weaker than that obtained with an equal amount of dextrose. For our purpose, we considered this a small enough error to be neglected. In presenting the results the word glucose is used for what is really, in some cases, a mixture of dextrose and fructose.

† We have not attempted to quantitate the degree to which the oxygen supply must be reduced to develop the rise in osmotic pressure but we do know that when it was reduced to such a degree that if prolonged it would have resulted in death, the rise invariably occurred.

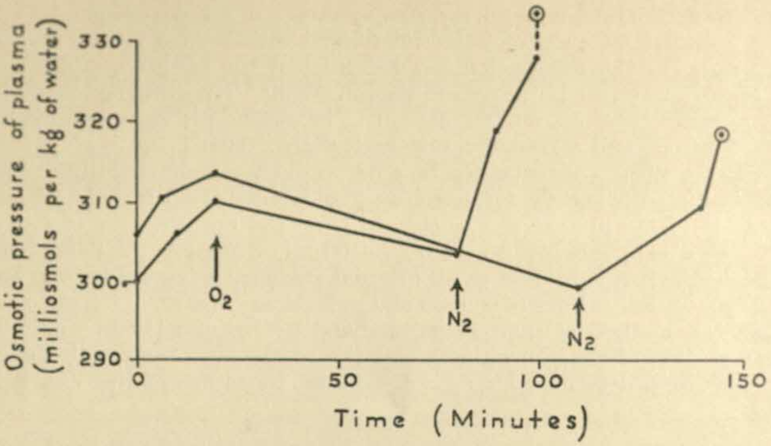

FIG. 1.—At time zero the two rabbits started to breathe nitrogen. Twenty minutes later they were allowed to breathe air again. At 80 and 110 min. respectively they started once again to breathe nitrogen. The points enclosed in a circle indicate that the samples were taken immediately after the heart had ceased beating.

TABLE I.—EXPERIMENT 9/22/56—RABBIT

Observed Values

Sample No.	1	2	3	4
Min. after onset hypoxia	0	20	40	100*
Total osmotic pressure (mOs/kg. H_2O)	298·2	304·8	319·5	312·8
Art. plasma:				
Glucose (mOs/kg. H_2O)	16·4	25·7	41·3	43·8
Na^+ (mOs/kg. H_2O)	142·5	140·5	139·2	133·5
K^+ (mOs/kg. H_2O)	4·4	4·9	5·0	5·1
Protein (g./100 ml.)	5·5	5·4	5·2	5·0
Art. O_2 (mM/l.)	5·9	1·8	1·1	5·6
Art. % sat.	100·0	14·0	20·0	100·0
Art. CO_2 (mM/l.)	25·6	16·2	8·6	16·4
Art. pH	7·45	7·46	7·26	7·37

Calculated Values

Art. plasma:				
C^+ (mOs/kg. H_2O)	146·9	145·4	144·2	138·6
A^- (mOs/kg. H_2O)	132·1	130·8	131·3	125·6
P^- (mOs/kg. H_2O)	14·8	14·6	12·9	13·0

Osmotic Balance

Samples compared	$\Delta\pi$	$=\Delta$Glucose	$+\Delta Na^+$	$+\Delta K^+$	$+\Delta A^-$	$+\Delta X$
2–1	+ 6·6 =	+ 9·3	− 2·0	+ 0·5	− 1·4	+ 0·1
3–1	+21·3 +	+24·9	− 3·3	+ 0·6	− 0·8	− 0·1
4–1	+14·6 =	+27·4	− 9·0	+ 0·7	− 6·5	− 2·0

C^+ = total cation, $A^- = C^+ - P^-$, P^- = anion equivalent of protein.
* Sample 4 taken 60 min. after animal began to breathe room air again.
mOs/kg. H_2O = milliosmols per kg. of water.

interval the glucose concentration rose another 2·5 whilst the concentration of the ions decreased 12·1 mOs.

The results of experiment 1/10/57 on a lamb (Table II) stand in contrast to those of experiment 9/22/56, for in the lamb virtually the entire rise in the osmotic pressure of the plasma can be ascribed to an elevation of the ions whose concentrations we have estimated. In the 40 min. period of hypoxia the osmotic pressure of the plasma of this lamb rose from 309·6 to 322·8 mOs.

TABLE II.—EXPERIMENT 1/10/57—LAMB—5 DAYS OLD

Observed Values

Sample No.	1	2	3
Min. after onset hypoxia	0	20	40
Total osmotic pressure (mOs/kg. H_2O)	309·6	320·3	322·8
Art. plasma:			
Glucose (mOs/kg. H_2O)	6·30	5·80	2·47
Na^+ (mOs/kg. H_2O)	151·8	154·0	157·6
K^+ (mOs/kg. H_2O)	3·85	5·30	4·90
Protein (g./100 ml.)	7·3	7·3	7·2
Art. O_2 (mM/l.)	4·46	0·9	0·2
Art. % sat.	78·0	15·0	3·4
Art. CO_2 (mM/l.)	26·9	13·1	9·4
Art. pH[2]	7·38	7·12	7·09

Calculated Values

Art. plasma:			
C^+ (mOs/kg. H_2O)	155·6	159·3	162·5
A^- (mOs/kg. H_2O)	136·4	142·3	146·0
P^- (mOs/kg. H_2O)	19·2	17·0	16·5

Osmotic Balance

Samples compared	$\Delta\pi$	$= \Delta$ Glucose	$+ \Delta Na^+$	$+ \Delta K^+$	$+ \Delta A^-$	$+ \Delta X$
2–1	$+10{\cdot}7 =$	$-0{\cdot}5$	$+ 2{\cdot}2$	$+ 1{\cdot}5$	$+ 5{\cdot}9$	$+ 1{\cdot}6$
3–1	$+13{\cdot}2 =$	$-3{\cdot}8$	$+ 5{\cdot}8$	$+ 1{\cdot}1$	$+ 9{\cdot}6$	$+ 0{\cdot}5$

C^+ = total cation, $A^- = C^+ - P^-$, P^- = anion equivalent of protein.

All but 0·5 mOs (ΔX) of this elevation is the algebraic sum of the rise in the concentration of ionic substances of the plasma of 16·5 and a decrease in glucose concentration of 3·8 mOs.

In other cases the rise in the osmotic pressure of the plasma associated with hypoxia was due to changes intermediate between these two extremes—that is to elevations of equal or varying amounts in the concentrations of the glucose and ionic components. Thus in one experiment (Table III) the osmotic pressure rose 36·0 mOs—290·0–326·0 in 50 min. of hypoxia. Calculation of the osmotic balance demonstrates that the rise was due to an elevation in glucose concentration of 14·7 mOs and one of 23·4 in the ions, the ΔX in this experiment being −2·4 mOs, well within experimental error.

In the three experiments described above the changes in the concentrations of the plasma components followed, *i.e.*, glucose, sodium, potassium and the anions, account—with but small remainders—for the elevation in the osmotic pressure of the plasma associated with hypoxia. Of the thirteen experiments for which a balance sheet was drawn the elevation could be similarly accounted for in five; in the remainder about 30 per cent of the elevation appeared to be due to changes in the concentration of other plasma components, *i.e.*, ΔX in our calculations of osmotic balance. In some cases ΔX and the change in the concentration of the ions seemed to account for the rise; for example in experiment 1/15/57 on a sheep (Table IV) the total osmotic pressure of the plasma rose in 84 min. of hypoxia from 296·2 to 328·0—an increase of 31·8 mOs. Of this elevation only 1·1 mOs appears to be due to a rise in the glucose concentration, 23·1 to an increase in the concentration of the ions, leaving 7·6 mOs assigned to changes in the concentration of unknown components. In another experiment on a rabbit 10/20/56 (Table V) the total osmotic pressure of the plasma rose 46·4 mOs in a 105-minute period of hypoxia from 290·5 to 336·9. Of this rise 28·8 mOs were due to an increase in plasma glucose, 3·6 to a rise in the ion concentration leaving 14·0 mOs unassigned.

TABLE III.—EXPERIMENT 1/13/57—LAMB—8 DAYS OLD

Observed Values

Sample No.	1	2	3	4
Min. after onset hypoxia	0	20	40	50
Total osmotic pressure (mOs/kg. H_2O)	290·2	310·2	321·4	326·0
Art. plasma:				
Glucose (mOs/kg. H_2O)	7·13	14·0	17·2	21·2
Na^+ (mOs/kg. H_2O)	139·4	145·3	146·5	147·2
K^+ (mOs/kg. H_2O)	4·28	4·65	4·83	6·72
Protein (g./100 ml.)	4·9	5·1	5·3	5·3
Art. O_2 (mM/l.)	5·2	0·5	0·6	2·1
Art. % sat.	72·0	7·0	8·0	28·0
Art. CO_2 (mM/l.)	22·4	23·4	26·6	25·9
Art. pH	7·32	6·57	6·44	6·46

Calculated Values

Art. plasma:				
C^+ (mOs/kg. H_2O)	143·7	149·9	151·3	153·9
A^- (mOs/kg. H_2O)	131·2	141·1	142·9	145·4
P^- (mOs/kg. H_2O)	12·5	8·8	8·4	8·5

Osmotic Balance

Samples compared	$\Delta\pi$	$=\Delta$ Glucose	$+\Delta Na^+$	$+\Delta K^+$	$+\Delta A^-$	$+\Delta X$
2–1	+20·2 =	+ 6·9	+ 5·9	+ 0·4	+ 9·9	− 2·9
3–1	+31·4 =	+10·1	+ 7·1	+ 0·5	+ 11·7	+ 2·0
4–1	+36·0 =	+14·1	+ 7·8	+ 2·4	+ 14·2	− 2·5

C^+ = total cation, $A^- = C^+ - P^-$, P^- = anion equivalent of protein.

In fig. 2 the percentage of the rise in osmotic pressure of the plasma associated with hypoxia due to the glucose is plotted against the percentage of the rise due to the electrolytes, *i.e.*, the sum of the plasma concentrations

TABLE IV.—EXPERIMENT 1/15/57—SHEEP

Observed Values

Sample No.	1	2	3
Min. after onset hypoxia	0	43	84
Total osmotic pressure (mOs/kg. H_2O)	296·2	300·8	328·0
Art. plasma:			
Glucose (mOs/kg. H_2O)	9·8	13·7	10·9
Na^+ (mOs/kg. H_2O)	149·5	149·5	156·2
K^+ (mOs/kg. H_2O)	3·85	4·15	7·45
Protein (g./100 ml.)	6·5	6·5	6·4
Art. O_2 (mM/l.)	4·0	2·2	0·5
Art. % sat.	51·0	27·0	6·0
Art. CO_2 (mM/l.)	24·2	19·8	8·7
Art. pH	7·50	7·47	7·11

Calculated Values

Art. plasma:			
C^+ (mOs/kg. H_2O)	153·3	153·6	163·6
A^- (mOs/kg. H_2O)	135·4	136·0	148·7
P^- (mOs/kg. H_2O)	17·9	17·6	14·9

Osmotic Balance

Samples compared	$\Delta\pi$	$= \Delta$ Glucose	$+ \Delta Na^+$	$+ \Delta K^+$	$+ \Delta A^-$	$+ \Delta X$
2–1	+ 4·6 =	+3·9	+ 0·0	+ 0·3	+ 0·6	− 0·2
3–1	+31·8 =	+1·1	+ 6·7	+ 3·6	+ 13·3	+ 7·1

C^+ = total cation, $A^- = C^+ - P^-$, P^- = anion equivalent of protein.

TABLE V.—EXPERIMENT 10/20/56—RABBIT

Observed Values

Sample No.	1	2	3	4
Min. after onset hypoxia	0	20	40	105
Total osmotic pressure (mOs/kg. H_2O)	290·5	290·5	298·8	336·9
Art. plasma:				
Glucose (mOs/kg. H_2O)	11·5	14·2	22·5	40·3
Urea (mOs/kg. H_2O)	8·3	9·4	9·7	9·2
Na^+ (mOs/kg. H_2O)	140·1	139·0	137·0	137·5
K^+ (mOs/kg. H_2O)	3·20	3·12	2·90	4·95
Protein (g./100 ml.)	6·2	6·2	6·0	6·0
Art. O_2 (mM/l.)	5·6	2·3	1·9	0·3
Art. % sat.	91·0	41·0	33·0	2·0
Art. CO_2 (mM/l.)	22·0	17·8	12·0	3·2
Art. pH	7·46	7·67	7·52	6·81

Calculated Values

Art. plasma:				
C^+ (mOs/kg. H_2O)	143·3	142·1	139·9	142·4
A^- (mOs/kg. H_2O)	126·5	123·9	123·3	130·5
P^- (mOs/kg. H_2O)	16·8	18·2	16·6	11·9

Osmotic balance

Samples compared	$\Delta\pi$	$= \Delta$ Glucose	$+ \Delta Na^+$	$+ \Delta K^+$	$+ \Delta A^-$	$+ \Delta$ Urea	$+ \Delta X$
2–1	+ 0·0 =	+ 2·7	− 1·1	− 0·1	− 2·6	+ 1·1	+ 0·0
3–1	+ 8·3 =	+11·0	− 3·1	− 0·3	− 3·2	+ 1·4	+ 2·5
4–1	+46·4 =	+28·8	− 2·6	+ 1·7	+ 4·0	+ 0·9	+13·6

Legend as in Table IV.

of sodium, potassium and osmotically active anions. So arranged the results of our experiments indicate that in the rabbits the rise in total osmotic pressure is due primarily to a rise in the plasma glucose, whereas in the sheep, lambs and fetuses the principal component in the rise was the change in the electrolyte concentration of the plasma. As this difference in the character of the response appeared to be due to a difference in the capacity of these two species to elevate their blood sugar in response to stress we carried out two experiments on rabbits from whom food had been withheld for 24 and 72 hr. respectively. The results of these two experiments were similar, the data present in experiment 12/15/56, Table VI are those obtained on the rabbit that had not fed for 72 hr.

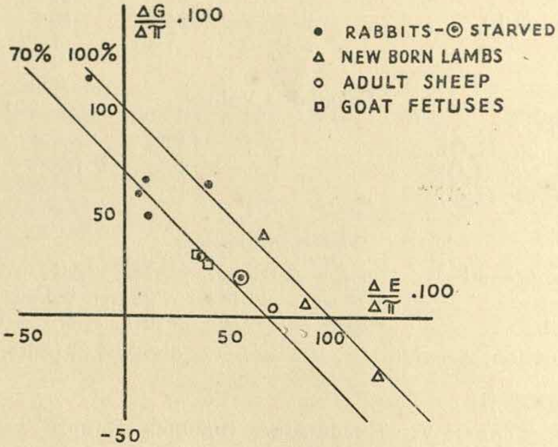

FIG. 2.—The percentage of the rise in osmotic pressure due to glucose is plotted against the percentage of the rise due to the electrolytes.

In that experiment the osmotic pressure of the plasma rose 24·5 mOs in the 40 min. period of hypoxia—from 298·5 to 323·0 whilst the glucose concentration increased but 5·1—this in contrast with increases ranging from 13·0 to 35·8 mOs in the rabbits that had fed *ad libitum* before exposure to hypoxia. The electrolyte concentration as estimated in this case rose 13·4 mOs leaving 6·0 mOs to be assigned to an unidentified concentration change—ΔX. This pattern of plasma response to hypoxia, in which the major contribution to the osmotic pressure rise is achieved by the ionic components of the plasma is characteristic of both sheep and goat species at all stages of development, from fetus to adult. In the well-fed rabbit, on the other hand, the marked hyperglycemia of hypoxia assumes greater importance. As to the ΔX, we were unable to find any correlation between the appearance of this unaccounted fraction and some variation in our experimental procedure. Urea concentration, determined in the two experiments with the largest unaccounted fraction, was found to be only slightly elevated, with a maximum increase of 2 mOs/kg. H_2O.

TABLE VI.—EXPERIMENT 12/15/56—RABBIT (3 DAYS STARVATION)

Observed Values

Sample No.	1	2	3
Min. after onset hypoxia	0	20	40
Total osmotic pressure (mOs/kg. H_2O)	298·5	312·1	323·0
Art. plasma:			
Glucose (mOs/kg. H_2O)	17·6	22·0	22·7
Na^+ (mOs/kg. H_2O)	147·2	146·5	149·7
K^+ (mOs/kg. H_2O)	2·5	4·5	5·5
Protein (g./100 ml.)	5·9	5·7	5·7
Art. O_2 (mM/l.)	6·3	1·7	1·7
Art. % sat.	78·0	9·0	9·0
Art. CO_2 (mM/l.)	19·5	12·8	12·4
Art. pH	7·25	7·16	6·94

Calculated Values

Art. plasma:			
C^+ (mOs/kg. H_2O)	149·7	151·0	155·2
A^- (mOs/kg. H_2O)	135·1	137·5	143·0
P^- (mOs/kg. H_2O)	14·6	13·5	12·2

Osmotic Balance

Samples compared	$\Delta\pi$	$= \Delta$ Glucose	$+ \Delta Na^+$	$+ \Delta K^+$	$+ \Delta A^-$	$+ \Delta X$
2–1	+13·6 =	+4·4	− 0·7	+ 2·0	+ 2·4	+ 5·5
3–1	+24·5 =	+5·1	+ 2·5	+ 3·0	+ 7·9	+ 6·0

C^+ = total cation, $A^- = C^+ - P^-$, P^- = anion equivalent of protein.
* Sample 4 taken 60 min. after animal began to breathe room air again.
mOs/kg. H_2O = milliosmols per kg. of water.

TABLE VII.—EXPERIMENT 9/11/56—RABBIT

Observed Values

Sample No.	1	2
Min. after intravenous glucose*	0	5
Total osmotic pressure (mOs/kg. H_2O)	291·9	305·7
Art. plasma:		
Glucose (mOs/kg. H_2O)	9·8	45·6
SC (mhos × 10^3)	12·32	11·68
Protein (g./100 ml.)	5·9	4·8
Art. O_2 (mM/l.)	5·6	5·6
Art. CO_2 (mM/l.)	29·0	21·9
Art. pH	7·47	7·30

Calculated Values

Art. plasma:		
C^+ (mOs/kg. H_2O)	148·8	139·8
A^- (mOs/kg. H_2O)	132·7	127·7
P^- (mOs/kg. H_2O)	16·1	12·1

Osmotic Balance

Samples compared	$\Delta\pi$	$= \Delta$ Glucose	$+ \Delta C^+$	$+ \Delta A^-$	$+ \Delta X$
2–1	+13·8 =	+35·8	− 9·0	− 5·0	− 8·0

* Intravenous glucose contained 6 g. glucose in 16 c.c. H_2O.
SC = specific conductivity of plasma in mhos × 10^3.
Remaining symbols as in Table VI.

In order to assess the rôle of the increase of the blood-sugar associated with hypoxia on the concomitant changes in the electrolyte concentration of the plasma we tested the affect of a rapid rise in the blood glucose on the electrolyte concentration in the absence of hypoxia. In experiment 9/11/56 (Table VII) 6 g. of glucose were given intravenously in 16 ml. distilled water to a well oxygenated rabbit after control samples had been taken. Five minutes later a second plasma sample was obtained. In the 5 min. interval the total osmotic pressure of the plasma rose 13·8 mOs, the glucose concentration 35·8 whilst the concentration of the electrolytes fell 15·4 mOs and ΔX the concentration of osmotically active but unidentified components fell 6·6 mOs. The results of this experiment stand in contrast with those of 9/22/56 (Table I) in which during hypoxia the glucose concentration rose 24·9 mOs, whilst the net concentration of the electrolytes fell but 3·6 mOs.

DISCUSSION

The data presented above demonstrate quite clearly that exposure to acute, severe hypoxia is accompanied by a rise in the osmotic pressure of the plasma in adult sheep and rabbits and new-born lambs as we had observed earlier to be the case in sheep and goat fetuses; they further indicate that the rise is in each case the integrated result of variations in the concentration of a number of plasma components that are combined in patterns which differ according to the species, age, state of nutrition, etc. of the animal.

From a study of these variations and the patterns in which they are combined some inferences may be drawn with regard to the possible mechanisms through which hypoxia initiates the observed changes in the plasma constituents. The following considerations are offered toward that end. In our interpretation of the data we have made one assumption *i.e.*, that the osmotic pressures of the intra- and extracellular fluids tend to equality.

If this assumption is valid the fall in the electrolyte concentration that accompanies the rise in the osmotic pressure due to the intravenous administration of glucose to a well-oxygenated rabbit (experiment 9/11/56—Table VII) can be ascribed to a shift of water from the intra- to the extracellular compartment, that dilutes the extracellular electrolytes, elevates the intracellular total osmotic pressure and restores the osmotic balance between the two fluid compartments.

However, as indicated by our data, an elevation in plasma glucose with hypoxia is not accompanied by the characteristic fall in plasma electrolytes seen in the well oxygenated animal—more commonly their concentration increases. How does tissue hypoxia alter this response to an elevation in plasma glucose? On the basis of our observations two possible explanations may be considered.

First, there is the possibilily that an alteration in tissue metabolism enforced by hypoxia results in an increase in the concentration of osmotically active components within the affected cells. The newly formed particles would have a tendency to move out into the extracellular fluids, and water to move in. The less freely diffusible these particles were, the more water would move in before an osmotic steady state would be re-established. However, if during the hypoxia the glucose concentration of the extracellular fluids rose at the same rate at which the intracellular concentration of osmotically active components, the osmotic balance between intra- and extracellular fluids would be re-established at a higher level with no shift of water, and therefore no change in the concentration of the plasma electrolytes. Intermediate conditions between these two extremes might be expected in which the osmotic balance upset by hypoxia would be restored in part through a rise in plasma glucose and in part through a movement of water from the extra- to the intracellular compartment and an increase in the concentration of the extracellular electrolytes. Thus in the intermediate conditions a reciprocal relationship would be expected between the rise in the concentrations of the plasma glucose and electrolytes.

The results of the experiments in which changes in the osmotic pressure could be accounted for by changes in the concentrations of plasma electrolytes and glucose—they fall on the 100 per cent line in fig. 2—are in good agreement with the suggestion advanced *i.e.*, that the number of osmotically active molecules of the intracellular compartment increases in hypoxia as a consequence of alterations of tissue metabolism. Thus experiment 1/10/57 (Table II) could illustrate the condition in which the osmotic balance disturbed by rise in the osmotic pressure of the intracellular compartment is restored by a movement of fluid into that compartment and a rise in extracellular electrolyte concentration. Experiment 9/22/56 (Table I) would represent the circumstances in which the glucose concentration in the extracellular fluids rises *pari passu* with the osmotic pressure of the intracellular compartment. The results of experiment 1/13/57 (Table III) may be taken to illustrate the condition in which the osmotic imbalance created by the rise in the osmotic pressure of the intracellular compartment is restored in part by a movement of water into it from the extracellular with a resultant rise in plasma electrolytes and in part by the elevation of the blood-sugar in response to the stress of hypoxia.

The experiments in which changes in the concentration of the plasma electrolytes and glucose do not completely account for the change in the osmotic pressure resulting from hypoxia suggest that other substances besides those that we have measured rise in their plasma concentration. Certainly part of the discrepancy between the measured rise in osmotic pressure and the calculated value can be attributed to analytical error and to the approximations used in calculating the electrolyte concentration in terms of mOs/kg. H_2O. However, a discrepancy of ten or more mOs, such as occurred in some experiments, appears to be outside the limits of experimental error and to indicate the change in concentration of one or more unknown substances.

The tendency for the points not on the 100 per cent line (fig. 2) to be distributed about the 70 per cent line suggests that when the concentration of this unknown component or components is affected in hypoxia, it tends to be responsible for about 30 per cent of the rise in the osmotic pressure. The nature of this plasma component or components is currently under investigation; this much we do know: that it is not represented in the non-protein nitrogen of the plasma.

In this connection the report of Rubin *et al.* [1956] on the total osmolality of the plasma of critically ill patients is of interest. They observed that in seventy-eight such patients, the osmolality measured exceeded the value calculated on the electrolyte concentration by 40–125 mOs/kg. H_2O and that the difference could not be entirely accounted for by urea nitrogen, glucose, protein or other commonly measured plasma constituents.

In the foregoing discussion one assumption inherent in the argument is that the total quantity of electrolyte in the extracellular compartment is constant and not directly altered by tissue hypoxia. But it must be recognized that an increase in the total extracellular electrolyte could account for the results we have obtained and must be considered as an alternative to the possibility of a rise in the number of osmotically active particles in the intracellular compartment brought about by an alteration in the character of tissue metabolism in hypoxia. The total electrolyte in the extracellular compartment might be increased from some store as a result of hypoxia much as the glucose concentration appears to be elevated in some animals.

Finally, whatever the explanation of the mechanisms through which the osmotic pressure of the plasma is elevated in response to hypoxia there is no evidence in our data to indicate that they differ fundamentally in the animals studied—the rabbit, sheep, lamb, and goat fetuses. Such differences as there are, appear to be in the degree to which the individual variables are adjusted rather than in the variables employed.

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THE REPRESENTATION OF THE RETINA ON THE OPTIC LOBE OF THE FROG. By R. M. GAZE. From the Physiology Department, Edinburgh University.

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The projection of the frog's retina to the optic lobe was studied by the use of a small optical stimulus and electrical recording from the lobe. A localized point-to-point representation was demonstrated.

THERE is a point-to-point representation of the retina on the superior colliculus in the cat [Apter, 1945] and in the monkey and goat [Cooper, Daniel and Whitteridge, 1953]. In birds and lower vertebrates the main visual centre is the optic lobe, which is homologous with the superior colliculus in mammals [Cajal, 1955]. Here, too, a systematic projection of the retina on the optic lobe has been found in pigeons [Hamdi and Whitteridge, 1954], fishes [Buser and Dussardier, 1953] and frogs [Buser, 1955].

In the experiments described here the retinal representation on the frog's optic lobe was studied in greater detail to find out the degree of localization present. A preliminary report of this work has already appeared [Gaze, 1956].

METHODS

The retinal representation was examined by use of a small visual stimulus and electrical recording from the optic lobe. In the first experiments frogs were anæsthetized with ethyl chloride followed by ether; in later experiments, by an injection of "Dial" liquid compound (Ciba) into the dorsal lymph space in dosage of 0·5 ml./kg. The top of the skull was opened and the brain anterior to the optic lobes removed by suction; this could usually be done without injury to the optic nerves, which pass under the cerebral hemispheres. The meningeal membrane was then stripped from one optic lobe and the cavity filled with liquid paraffin. The animal was then pinned securely to a board and the effect of the anæsthetic allowed to wear off. This took about an hour. Animals so treated were found in most cases to remain motionless for between 3–5 hr. If movements were seen curarine was given in dosage of 10 mg./kg.

Action potentials were recorded from the optic lobe in most experiments by monopolar steel micro-electrodes (tip diameter between 5 and 20 μ). In a few experiments recording was by glass micro-pipettes filled with 3M KCl (tip diameter about 1 μ). It was found that for surface recording the steel electrodes gave better results than the micro-pipettes. The recording circuit was completed by an indifferent electrode on one of the hindlimbs. Action potentials were amplified by an RC coupled amplifier (overall time constant approximately 5 msec.) fitted with a loudspeaker and were displayed on an oscilloscope.

The stimulating light source was the flash of a miniature neon discharge tube which was driven by exponential pulses from a stimulator unit. The stimulus was locked to the time base and arranged to recur at approximately 1 sec. intervals.

The neon tube could be moved on a perimeter arm and the angular size of the stimulus could be varied by altering the aperture in front of it. In most of the experiments the aperture subtended 15 minutes of arc at the eye; in some specified instances an aperture of 30 minutes of arc was used; and in a few experiments the neon was used naked, in which case it subtended some 2° at the eye. The pulses supplied to the neon tube were not altered in any way, the aperture alone being varied. The proper centering of the perimeter on the eye caused some difficulty; the frog retina has no fovea [Walls, 1942] and centering was done by observing the reflexion of a spot of light from the cornea. Light from a distant source was reflected on to the eye by a mirror ophthalmoscope held at the centre of the perimeter arc. When the light reflected from the cornea was seen (through the perimeter and ophthalmoscope) to be in the centre of the frog's pupil, the perimeter was considered to be centred. This method, although the best that could be devised, is crude, and probably permits errors of several degrees in the estimation of the centre point of the visual field. While most of the recordings were done in a darkened room, control experiments were carried out in daylight.

RESULTS

With a steel micro-electrode touching the surface of the lobe a response consisting of a brief burst of action potentials from several different units, could be obtained following stimulation by the neon flash in the appropriate part of the opposite visual field. In some cases action potentials from a single unit stood out even when these relatively large electrodes were used. With the electrode recording from a point on the lobe surface, the response could only be evoked by stimulation within a certain localized area in the opposite visual field. The size of this effective area was as small as 5° radius with some responses and as large as 60° radius with others. Increasing the diameter of the stimulus light (from 15 to 30 minutes of arc) made the effective area of visual field larger. In a few instances the width of receptive field for single unit responses was measured and one such response area was found to cover 20° of visual field. When a multi-unit response was obtainable from a large area of field there was usually some position of the stimulus within this area which gave the biggest response at the lobe; and this position was the one then used for mapping the retinal projection. With dark-adapted frogs, and using a stimulus 15 minutes of arc in diameter, no systematic difference in size has been found between response areas at the periphery and those towards the centre of the visual field. With light-adapted frogs, and using a stimulus 2° in diameter, similar results were obtained. The latency of the visual responses at the optic lobe has ranged in different experiments from 105 to 340 msec.

Representation of the Retina on the Optic Lobe.—Points on the naso-inferior, quadrant of the retina projected to the posterior part of the lobe near the midline; points on the temporo-inferior retina projected to the anterior part of the lobe near the midline; points on the naso-superior retina projected to the posterior part of the lobe near its lateral edge while those on the temporo-superior retina projected to the anterior part of the lobe near its lateral edge.

Some of these relations are shown in fig. 1. In this experiment the upper surface of the optic lobe was explored thoroughly with the micro-electrode; the stimulus positions corresponding to the various electrode positions are shown on the perimeter chart for the opposite eye. Much the greater part of the upper lobe surface was taken up with the representation of the upper visual field. Stimulus positions 11, 6, 16 and 19 all lie on the 90° circumference of the visual field; that is, they are all 90° distant from the centre point.

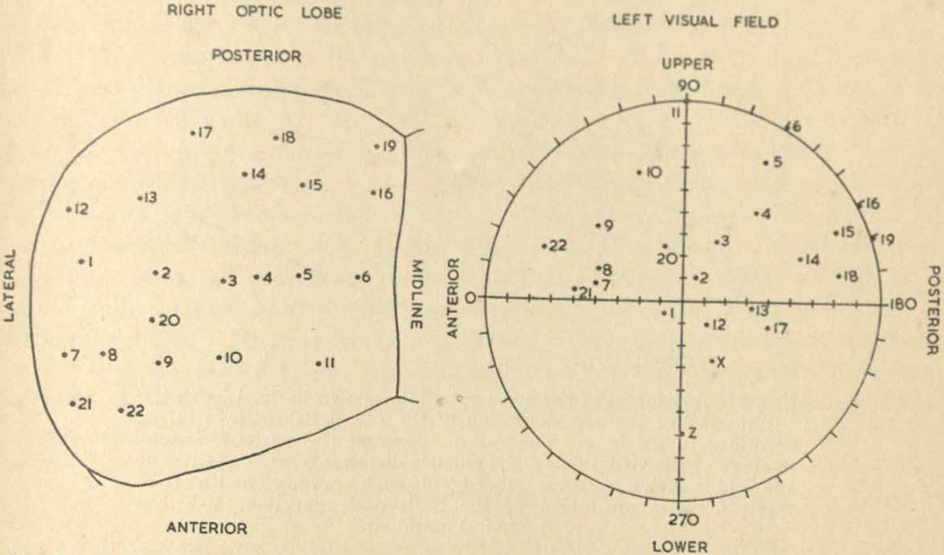

FIG. 1.—The surface of the right optic lobe was explored thoroughly with a steel micro-electrode. The numbered dots on the lobe diagram indicate the electrode positions and the corresponding numbers on the perimeter chart show the optimal stimulus positions in the contralateral visual field. Positions *x* and *z* in the visual field are described in the text.

The optic lobe is represented as seen from above and the visual field as seen from in front of the eye.

Similarly, stimulus positions 14, 4, 9 and 21 all lie approximately on the 50° circumference. If, on a diagram of the lobe surface, the appropriate electrode positions are connected by lines, a "contour map" is obtained of the visual field as it projects on to the lobe. This has been done for the experiment illustrated in fig. 1 and the result is shown in fig. 2.

From fig. 1 it will be seen that, while the electrode positions are well distributed over the upper surface of the lobe, the corresponding stimulus positions are mostly confined to the upper half of the visual field. This suggests that some of the representation of the lower visual field lies on that part of the optic lobe which curls round under its lateral edge. Support for this idea came from the same experiment; when the mapping of the upper lobe surface had been completed, the electrode was placed for a second time on lobe position 12 and lowered through the tissue so as to traverse the lateral edge of the lobe. With the electrode tip on the surface, response occured from position 12 in the visual field; with the tip at depth 0·23 mm., response

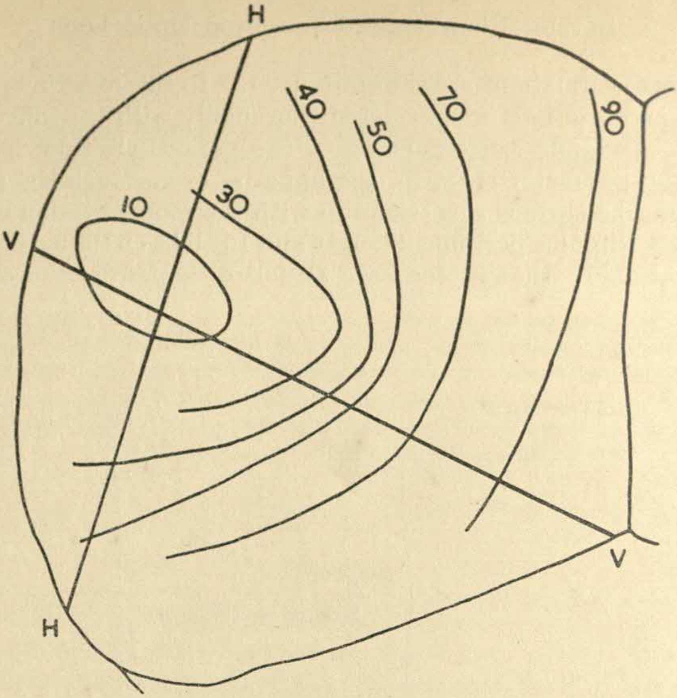

Fig. 2.—A diagram of the same optic lobe shown in fig. 1, with the "contours" of the representation of the visual field drawn in, as described in the text. The curves represent the various circumferences of the visual field; the angular distance from the centre of the field is shown by the number beside each curve. The line H-H represents the projection of the horizontal meridian, V-V the vertical meridian.

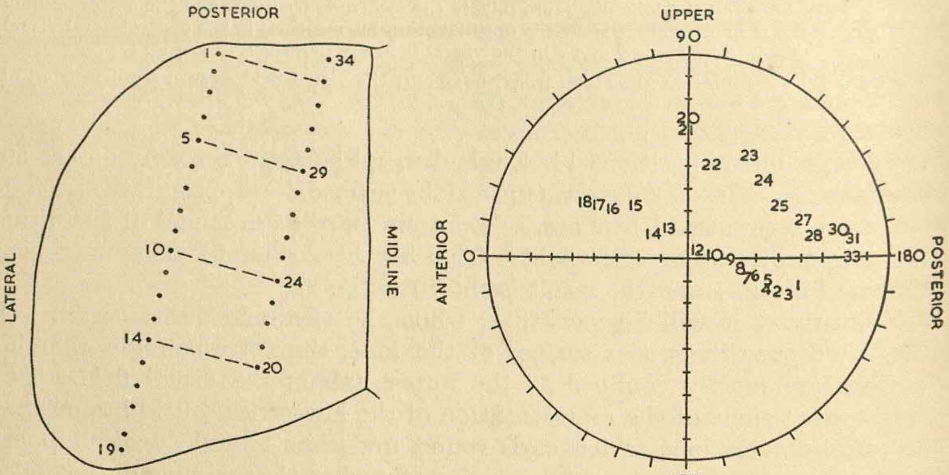

Fig. 3.—The change in optimal stimulus position which occurs on movement of the recording electrode over the surface of the optic lobe. The dots on the lobe diagram from 1 to 19 and from 20 to 34 represent electrode positions on the lobe and the numbers on the perimeter chart represent optimum stimulus positions as described in the text. Stimulus positions 11, 26, 29, 32 and 34 have been omitted. The parallel rows of electrode positions are 0·5 mm. apart. The dashed lines connect positions that are opposite each other on the two rows.

was obtained from position x in the visual field; and when the tip was at depth 1·75 mm., response was obtained from position z.

The precision of the retinal representation can be seen better in fig. 3. In this experiment the recording electrode was moved across the lobe from back to front in steps of 0·1 mm., as shown by the positions on the lobe diagram from 1 to 19. As the electrode was moved to each new position the optimum position of the stimulus was found to move across the visual field, as shown by the corresponding numbers on the perimeter chart. At electrode position 19 no response was obtained so the electrode was moved to position 20 and thence in 0·1 mm. steps to position 34. The two rows of electrode positions were parallel to each other and separated by 0·5 mm. Position 20 was directly opposite position 14; 24 was opposite 10; 29 was opposite 5; these relations are shown on the diagram by dashed lines. On the perimeter chart some of the stimulus positions have been omitted for clarity; these all fell where they were expected in numerical sequence. From the results of this and similar experiments it has been estimated that, in dark-adapted frogs, movement of the electrode by 0·1 mm. across the surface of the lobe results in an average shift of optimum stimulus position of between 6° and 7° in the visual field. No systematic change has been found in this figure between central and peripheral parts of the visual field. This observation has been repeated in light-adapted animals, using a stimulus of 2° diameter, with similar results.

DISCUSSION

Two disadvantages of the experimental method deserve attention: firstly, the error inherent in the method used for locating the centre point of the visual field makes difficult the comparison of the retinal projection in one frog with that in another; secondly, the use of a brief neon flash as stimulus made it impossible to distinguish between "on", "off" and "on-off" responses.

These experiments confirm and extend the observations of Buser [1955], who showed that each retinal quadrant in the frog sent fibres to a different part of the optic lobe. The results of the present work reveal considerable precision in the retinal representation on the lobe, despite the large area of receptive field (5–60° radius) which may excite a point on the lobe. The latencies of unit responses in these experiments were considerably greater (over 100 msec. compared with 30–50 msec.) than those reported by Andrew [1955]. The experimental conditions were different in the two cases and no satisfactory explanation of the difference in latencies can be given.

It is interesting that the results given here do not suggest that any one area of the retina is more specialized than another in the sense of having either a greater representation on the lobe or a smaller receptive field. This negative finding accords with histological evidence that the frog retina has no fovea and only a poorly developed "area".

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THE RELATION BETWEEN ALVEOLAR OXYGEN PRESSURE AND THE RESPIRATORY RESPONSE TO CARBON DIOXIDE IN MAN.

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Ventilation, respiratory frequency and the alveolar gas pressures have been measured in the steady state in resting man breathing various gas mixtures. In confirmation of the results of earlier workers, the slope of the linear part of the relation between ventilation and carbon dioxide has been shown to be an inverse function of the alveolar oxygen pressure, and the form of this function is discussed. The intercept of the $\dot{V}$-pCO_2 line on the pCO_2 axis is independent of pO_2.

The relationships in the steady state between pulmonary ventilation ($\dot{V}$, l./min., B.T.P.S.) and the partial pressures of alveolar carbon dioxide (pCO_2) and oxygen (pO_2) have been observed in two ways. The $\dot{V}$-pCO_2 relation, which over a certain range is linear, has been investigated at several constant inspired pO_2's [Lindhard, 1911; Nielsen, 1936; Hall, 1953], and at a few constant alveolar pO_2's [Nielsen and Smith, 1951; Asmussen and Nielsen, 1957; Kellogg, Vaughan and Badger, 1957]. The $\dot{V}$-pO_2 relation, which is not linear, has been investigated at several constant pCO_2's [Cormack, Cunningham and Gee, 1957]. Both these approaches reveal positive interaction between the respiratory effects of the two gases, and fail to support the contention of Gray [1950] that these effects are simply additive.

The results of Nielsen and Smith indicated a simple relation between pO_2 and the slope of the $\dot{V}$-pCO_2 line, and the present work is partly concerned with a more detailed investigation of this relation. An attempt has been made to express $\dot{V}$ quantitatively as a function of pO_2 and pCO_2. The work has been briefly reported elsewhere [Lloyd, Jukes and Cunningham, 1956].

METHODS

The apparatus for supplying the subject with any mixture of oxygen, carbon dioxide and nitrogen, and for measuring $\dot{V}$ and the alveolar gas pressures has been fully described elsewhere [Cunningham, Cormack, O'Riordan, Jukes and Lloyd, 1957]. Appropriate checks were made on the method of end-tidal sampling, which was used alone in most experiments. Estimations of pCO_2 were made with the flowbridge analyzer and, with those of pO_2, in the Haldane apparatus.

The aim of any one experiment was to observe the linear part of the $\dot{V}$-pCO_2 steady-state relation at two or more pO_2's in a single session lasting about 3 hr. The pooling of results from different days on any one subject introduces some extra scatter and so each experiment was as far as possible self-contained. Ventilation was recorded on the drum paper and pCO_2 measured with the flowbridge analyzer throughout an experiment; the readings of the latter showed when the steady state had been reached and the samples for Haldane analysis could be taken. The changes in pCO_2 between steady states were kept as small as possible by the design of the experiments, in most of which 10–20 sets of measurements were made. The steady state is very rapidly reached after changes in pO_2 when pCO_2 is held constant [Cormack *et al.*, 1957].

The inspired gas mixtures needed to fulfil the plan of a given experiment were deduced from graphs based on the approximate relation:

$$\text{Inspired} - \text{alveolar gas concentration} \simeq \frac{\text{Metabolic exchange of gas}}{0{\cdot}8\ \dot{V}} \quad . \quad (1)$$

The approximate respiratory exchange of oxygen and carbon dioxide at various values of $\dot{V}$ was derived from Otis [1954], and the expected value of $\dot{V}$ for a given pair of values of pO_2 and pCO_2 was deduced from Nielsen and Smith [1951], Cormack *et al.* [1957] and our own body of data as they accumulated. The factor 0·8 is the approximate ratio of alveolar to total ventilation, which for this purpose may be treated as if it were constant.

The subjects were healthy males. Ages, weights and heights are given in Table I. The men were post-absorptive in all experiments except No. 13. They did not know the plan of the experiments and found it impossible to deduce what sort of mixture they were getting. Subjects 4 and 7 were subjects 4 and 7 of Cormack *et al.* [1957]. They found the present series of experiments more comfortable than the earlier series, and showed no periodic breathing or excessive hyperventilation, which may be signs of psychological interference. In the present series inspired gas mixtures were humidified, and an electric fire was used when necessary to keep the subject warm.

RESULTS

The full results of the experiments are given in Table I, in which each line presents the observations during the steady state on one inspired gas mixture. The lines are printed in the experimental sequence.

Fig. 1 gives scatter diagrams for six representative experiments, $\dot{V}$ being plotted against pCO_2. The lines fitted are the reduced major axes [Kermack and Haldane, 1950] at different pO_2's.

Table I.—Alveolar Gas Pressures (pCO_2, pO_2), Ventilation ($\dot{V}$) and Respiratory Frequencies (f) for all Experiments, together with Physical Characteristics of Subjects. Each Horizontal Line in the Table represents one Steady-state Determination. Determinations marked with Asterisks have been used for Calculation of the Reduced Major Axes. Bracketed Values for pCO_2 were determined with the Flowbridge Analyzer only; Bracketed Values for pO_2 were deduced from the Composition of the Inspired Mixture, and the Measured Ventilation and Alveolar pCO_2.

Subject 4, age 35 years, 64 kg., 171 cm.

Experiment 4

pCO_2	pO_2	$\dot{V}$	f
40·2	164·2	8·9	15·2 *
46·0	159·8	20·5	18·0 *
48·4	157·8	29·0	19·0 *
50·2	158·2	38·5	20·8 *
51·8	158·8	46·7	23·6 *
55·2	152·2	56·0	25·2 *
55·3	(152)	56·8	24·0 *
52·7	155·1	49·0	24·0 *
50·4	157·2	46·6	24·0 *
47·0	159·8	35·8	23·6 *
44·2	164·1	22·6	23·4 *
38·9	161·7	9·0	17·4 *
57·7	145·5	60·2	24·0 *
61·6	143·2	67·0	25·0 *
60·6	145·3	63·6	25·0 *

Experiment 5

pCO_2	pO_2	$\dot{V}$	f
40·2	153·3	10·8	16·4 *
45·5	162·6	19·9	17·2 *
48·3	157·5	27·9	19·0 *
53·0	153·1	46·2	24·8 *
51·2	101·7	44·8	24·8
51·9	82·1	50·2	28·0
49·9	59·0	51·3	25·3
48·5	156·2	29·1	23·6 *
47·7	86·2	31·4	24·2
47·0	65·4	32·5	24·4
45·0	45·5	35·0	25·0
51·2	152·0	39·2	24·2 *
51·6	410	39·0	24·2
51·2	460	40·1	26·2
45·7	350	25·9	22·8

Experiment 6

pCO_2	pO_2	$\dot{V}$	f
40·2	159·2	9·2	14·4 *
44·9	150·2	17·6	14·6 *
46·1	152·4	27·3	21·8 *
45·9	152·0	36·0	22·6 *
49·8	150·5	44·2	24·0 *
52·9	146·1	56·4	25·4 *
50·6	150·0	46·8	24·2 *
49·0	150·2	37·2	23·2 *
44·5	156·1	23·7	22·2 *
41·5	158·3	9·3	17·6 *

Experiment 7

pCO_2	pO_2	$\dot{V}$	f
39·2	165·0	9·8	17·0 *
47·5	157·7	27·4	19·0 *
51·7	153·2	43·9	22·2 *
51·4	103·5	46·7	24·2
50·9	81·6	44·6	23·4
50·9	61·9	55·4	25·2
46·2	38·3	76·3	28·2
49·7	155·5	37·0	23·6 *
49·0	86·1	44·2	25·6
47·5	48·7	64·2	28·4
45·9	72·2	34·2	26·0
44·2	158·0	22·9	23·8 *
55·5	150·6	55·1	24·6 *
58·8	145·2	67·4	26·4 *

Experiment 8

pCO_2	pO_2	$\dot{V}$	f
37·8	166·9	11·7	19·4 *
46·2	155·2	32·5	21·2 *
51·0	152·3	49·6	25·0 *
50·6	102·9	52·6	25·2
51·2	81·4	57·3	28·0
49·6	61·9	61·6	29·8 *
49·6	149·7	45·1	25·4 *

Experiment 12

pCO_2	pO_2	$\dot{V}$	f
42·4	47·7	17·0	17·8 *
41·6	38·7	25·1	20·4 *
39·7	37·8	24·9	20·2
41·2	41·8	10·6	17·0
40·3	102·0	10·4	19·0 *
43·8	98·5	17·8	18·4 *
42·6	42·2	32·8	21·0 *

TABLE I.—(*continued*)

Experiment 8—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
48·8	83·4	42·5	24·2
48·4	63·0	46·9	25·6 *
46·0	86·2	34·0	24·4
45·6	65·4	34·5	24·0 *
44·9	45·4	40·9	..
46·0	156·2	28·1	22·2 *
43·0	67·2	27·9	24·4 *
42·0	47·2	35·6	23·4
40·0	67·1	11·7	21·0 *
38·1	50·0	11·7	20·2

Experiment 12—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
41·7	39·6	35·2	23·6 *
43·4	49·0	27·3	21·8 *
42·8	42·2	45·1	23·8 *
45·5	49·1	42·6	23·6 *
45·2	101·0	35·3	24·0 *
45·4	49·3	57·2	25·5 *
47·4	54·1	62·9	27·0 *
47·5	99·4	45·4	24·6 *
51·8	99·4	62·0	25·4 *

Subject 20, age 21 years, 75 kg., 180 cm.

Experiment 9

pCO_2	pO_2	$\dot{V}$	f
42·6	104·7	12·0	16·6 *
44·0	54·3	17·2	17·6 *
43·1	44·5	19·1	17·8 *
45·4	41·4	24·4	16·4 *
46·6	99·8	20·2	16·0 *
46·6	52·9	30·3	17·4 *
46·9	42·2	40·7	19·6 *
47·8	42·3	47·8	21·6 *
48·2	51·1	36·0	17·2 *
49·2	101·7	35·4	19·2 *
48·5	52·5	57·2	21·2 *
49·2	42·4	61·6	22·0 *
51·9	(100)	60·8	24·8 *
50·9	49·4	63·2	21·6 *

Experiment 10

pCO_2	pO_2	$\dot{V}$	f
44·7	42·6	19·6	15·8 *
46·6	41·3	26·0	18·0 *
45·4	46·9	18·4	16·6 *
46·7	41·4	30·4	18·6 *
46·4	50·6	27·3	19·0 *
46·6	99·8	21·4	18·8 *
46·1	41·0	48·0	21·6 *
47·2	51·2	48·0	24·0 *
46·8	98·6	38·1	22·2 *
47·4	42·2	67·4	27·2 *
48·2	48·8	59·8	26·2 *
48·2	97·7	47·8	24·4 *
50·6	48·3	77·2	28·2 *
52·1	100·0	52·4	25·2 *
53·4	97·2	61·4	25·8 *
43·6	51·5	31·1	20·8 *

Experiment 11

pCO_2	pO_2	$\dot{V}$	f
51·7	96·7	49·3	23·6 *
49·9	98·5	50·6	23·4 *
50·3	47·1	49·6	21·6 *
48·0	95·3	32·4	20·4 *
46·4	47·7	41·6	20·4 *
47·2	40·6	52·6	23·2 *
46·2	96·8	38·9	23·4 *
46·1	50·6	48·5	23·2 *

Experiment 11—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
45·8	39·4	53·7	22·8 *
44·4	99·0	29·3	21·0 *
45·8	48·3	30·4	16·4 *
44·7	40·9	45·2	23·8 *
42·8	48·9	25·8	21·8 *
44·5	46·3	20·5	17·2 *
45·6	41·1	37·0	19·6 *
43·7	40·8	26·2	18·0 *

Subject 21, age 20 years, 71 kg., 184 cm.

Experiment 13

pCO_2	pO_2	$\dot{V}$	f
58·8	98·6	56·2	28·8 *
56·4	101·5	54·0	28·2 *

Experiment 14

pCO_2	pO_2	$\dot{V}$	f
59·1	97·8	54·4	22·8 *
56·3	99·5	54·9	24·4 *

TABLE I.—(*continued*)

Experiment 13—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
55·9	100·7	48·9	26·6 *
55·5	63·8	63·8	30·0
53·1	102·8	41·2	24·6 *
51·7	49·3	58·0	28·4
52·8	98·5	31·0	19·6 *
48·7	101·0	27·7	20·8 *
48·0	45·7	66·2	31·4 *
47·3	44·9	54·7	27·0 *
47·2	45·1	44·3	26·2 *
46·4	99·6	19·2	20·4 *
46·3	44·5	49·0	24·8 *
45·2	46·1	40·1	26·0 *
45·0	47·4	39·1	25·4 *
42·7	45·8	27·5	26·2 *

Experiment 14—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
55·5	99·5	52·9	23·8 *
53·3	101·9	49·6	23·6 *
51·3	99·4	41·9	22·8 *
49·0	43·7	62·8	24·4 *
47·9	43·3	52·9	22·4 *
48·5	99·4	24·2	18·8 *
46·9	44·7	49·3	22·0 *
46·9	97·9	18·6	16·8 *
45·8	44·9	43·4	21·4 *
44·7	45·2	38·1	22·6 *
43·9	46·8	34·4	22·8 *
42·4	46·1	25·9	22·6 *

Experiment 15

pCO_2	pO_2	$\dot{V}$	f
58·1	99·6	67·2	23·4 *
56·3	100·6	62·8	20·6 *
54·1	101·4	57·5	23·8 *
52·0	103·7	50·8	23·2 *
50·4	101·1	41·7	23·2 *
46·8	45·8	59·2	22·2 *
45·9	45·7	56·2	22·8 *
45·9	102·9	36·9	21·8 *
45·6	45·2	57·5	22·0 *
44·0	45·5	45·7	21·2 *

Experiment 15—(*continued*)

pCO_2	pO_2	$\dot{V}$	f
43·7	102·3	30·5	22·6 *
44·2	46·7	43·6	20·6 *
41·1	44·2	32·3	22·6 *
44·0	60·7	36·8	25·8
43·5	46·0	44·5	25·2 *
44·2	71·3	36·0	23·4
42·6	72·0	26·6	23·8
48·7	65·1	69·7	23·6
42·4	58·1	43·6	22·6
40·3	50·0	23·8	23·4

Subject 22, age 22 years, 76 kg., 185 cm.

Experiment 16

pCO_2	pO_2	$\dot{V}$	f
58·8	96·7	54·2	18·2 *
56·4	99·4	51·3	19·2 *
54·8	99·4	51·2	19·6 *
54·6	99·7	36·6	14·6 *
51·1	98·3	38·4	17·4 *
49·6	97·5	22·9	10·2 *
45·7	101·4	25·5	17·8 *
42·6	49·1	26·0	18·8
42·5	44·6	30·8	13·6 *
44·5	44·1	35·9	16·0 *
44·9	44·2	38·4	17·2 *
45·5	43·1	42·1	16·0 *
46·2	43·8	45·0	16·0 *
46·0	45·1	63·3	21·0 *

Experiment 17

pCO_2	pO_2	$\dot{V}$	f
60·6	97·0	54·6	21·2 *
57·5	100·2	50·2	20·6 *
57·3	98·8	42·7	17·2 *
54·9	101·0	37·5	18·0 *
52·7	98·9	35·7	18·2 *
50·0	99·8	27·7	15·2 *
47·7	100·2	20·8	16·2 *
43·6	46·5	25·5	14·8
44·7	43·3	25·3	14·8 *
46·8	44·1	30·7	15·6 *
47·5	43·6	31·4	16·4 *
48·2	43·3	36·8	16·8 *
50·8	42·0	35·7	14·2 *
50·1	42·2	39·2	14·6 *
50·6	43·0	47·9	18·8 *
50·8	41·5	49·5	18·8 *

TABLE I.—(*continued*)

Experiment 18

pCO$_2$	pO$_2$	$\dot{V}$	f
51·6	48·3	58·6	25·0
49·3	47·5	49·4	21·0
45·1	41·9	44·1	23·6 *
45·0	41·1	31·9	18·6 *
45·2	39·8	29·5	17·4 *
41·4	42·2	27·9	14·4
48·1	92·4	15·8	14·8 *

Experiment 18—(*continued*)

pCO$_2$	pO$_2$	$\dot{V}$	f
49·7	94·8	22·6	15·2 *
51·0	97·1	40·4	17·2 *
53·8	99·1	48·8	17·4 *
58·1	95·7	55·6	18·6 *
45·4	43·3	91·3	29·8 *
44·6	41·9	57·0	24·0 *

Subject 23, age 23 years, 76 kg., 170 cm.

Experiment 19

pCO$_2$	pO$_2$	$\dot{V}$	f
58·7	98·1	52·0	30·8 *
56·3	100·3	71·0	40·0 *
54·7	100·8	67·8	42·4 *
47·8	101·1	30·6	28·8 *
50·4	100·2	39·5	31·2 *
52·1	102·7	47·2	38·4 *
55·3	99·1	51·6	35·2 *
46·6	99·7	19·4	24·4 *
44·0	44·0	21·3	22·6 *
44·8	41·8	22·9	23·6 *
46·4	41·8	25·6	24·2 *
46·3	43·4	29·4	27·6 *
46·7	43·5	38·4	36·0 *
47·2	44·1	44·5	39·8 *
48·1	44·5	61·2	54·5 *

Experiment 20

pCO$_2$	pO$_2$	$\dot{V}$	f
53·6	101·9	39·2	27·8 *
55·4	101·0	46·2	29·4 *
56·7	100·7	54·3	33·6 *
56·1	99·3	54·3	35·0 *
51·0	102·0	41·6	31·8 *
48·4	101·5	31·4	29·4 *
47·1	100·9	21·8	26·8 *
47·2	44·0	33·8	26·2
48·2	40·9	38·3	29·2 *
47·8	41·6	41·1	33·8 *
48·4	41·6	46·5	35·2 *
49·0	41·6	47·1	37·6 *
50·3	43·2	56·0	41·5
50·9	41·8	56·8	32·2 *
46·7	40·4	28·5	24·8 *
46·3	46·9	25·8	24·0

Subject 24, age 21 years, 76 kg., 190 cm.

Experiment 21

pCO$_2$	pO$_2$	$\dot{V}$	f
58·9	99·7	68·3	30·6 *
56·5	102·5	64·6	26·2 *
55·7	102·6	54·4	22·8 *
53·7	104·1	50·2	22·6 *
52·4	102·4	45·7	20·4 *
49·1	101·8	29·2	17·6 *
48·3	101·0	25·1	17·6 *
41·7	48·2	30·4	20·6
43·1	45·3	29·5	16·6 *
45·2	45·0	35·9	18·6 *
46·5	46·0	40·5	19·2 *
46·5	45·0	51·0	23·2 *
47·5	46·0	63·4	26·4 *
48·8	45·2	65·8	24·2 *

Experiment 22

pCO$_2$	pO$_2$	$\dot{V}$	f
59·5	99·8	72·7	28·6 *
(58·5)	(100)	72·5	27·0 *
56·3	102·0	63·0	27·2 *
54·7	103·4	51·5	18·8 *
52·8	101·1	44·7	20·0 *
49·9	102·7	35·7	16·0 *
48·0	102·2	27·9	17·4 *
42·8	46·0	34·4	17·2 *
44·1	44·2	27·6	15·6 *
45·3	45·3	33·7	15·6 *
45·8	46·7	39·7	17·2 *
46·4	45·1	53·6	18·8 *
47·3	45·7	68·7	21·8 *
47·6	45·9	75·6	26·8 *

TABLE I.—*(continued)*

Subject 25, age 22 years, 64 kg., 173 cm.

Experiment 23

pCO_2	pO_2	$\dot{V}$	f
52·4	86·4	50·6	25·4
53·0	94·9	56·0	28·6
49·2	94·5	45·8	28·4 *
47·2	94·2	34·3	27·8 *
47·6	96·6	27·6	19·0 *
44·4	44·9	26·8	23·0 *
44·7	46·8	30·1	23·4 *
46·9	45·5	36·7	19·2 *

Experiment 23—*(continued)*

pCO_2	pO_2	$\dot{V}$	f
46·4	44·9	41·3	23·2 *
46·9	44·7	52·2	29·6 *
44·9	40·3	47·5	32·4
44·6	41·5	47·8	25·4
42·5	41·0	44·2	25·8
42·4	40·6	39·1	25·8
42·3	41·8	38·2	24·2
54·2	91·5	62·4	29·4 *

Subject 7, age 26 years, 75 kg., 183 cm.

Experiment 24

pCO_2	pO_2	$\dot{V}$	f
55·4	106·5	64·8	27·2 *
52·9	105·5	59·4	30·8 *
48·8	107·0	50·0	31·3 *
43·2	108·5	38·0	28·2 *
38·9	108·5	14·6	31·6 *
39·0	54·4	20·7	26·0 *
42·1	55·2	34·9	30·2 *
45·5	54·2	51·2	33·2 *
47·7	53·7	70·2	36·8 *
43·0	45·4	37·1	22·6
44·5	44·9	49·0	26·6
42·3	47·0	36·4	21·2

Experiment 25

pCO_2	pO_2	$\dot{V}$	f
55·2	106·1	57·8	30·6 *
54·7	103·0	53·6	33·2 *
50·6	105·5	47·7	32·8 *
43·6	105·5	26·3	30·8 *
39·9	107·1	13·0	27·2 *
40·8	54·2	18·3	26·4 *
41·8	58·2	24·1	25·2 *
42·6	55·2	35·5	31·2 *
43·4	56·7	41·9	29·2 *
45·7	55·2	55·2	30·8 *
48·7	61·0	54·3	29·8 *
45·3	62·9	43·2	26·4 *
44·0	64·1	32·8	21·2 *
41·3	65·5	23·5	18·2 *
40·5	66·5	15·3	16·6 *

Experiment 26

pCO_2	pO_2	$\dot{V}$	f
47·1	142·1	20·7	17·0 *
47·2	101·5	25·3	18·0 *
47·0	72·8	26·0	17·2 *
45·9	58·6	29·5	18·4 *
45·7	51·2	32·4	19·4 *
45·5	45·3	39·4	21·0
46·7	48·9	46·8	24·2 *
43·1	144·6	12·2	13·4 *
42·7	99·5	13·2	14·6 *
42·6	75·1	13·8	14·8 *
42·2	62·0	15·6	15·6 *
41·5	54·0	18·3	16·8 *
41·9	48·8	22·0	16·6 *
41·5	42·9	24·2	18·2 *
45·3	41·4	51·6	23·8 *

Experiment 27

pCO_2	pO_2	$\dot{V}$	f
53·6	131·1	53·3	24·0 *
51·5	105·7	53·5	24·6 *
44·8	84·3	39·7	23·4 *
46·4	64·8	35·8	22·2 *
45·5	55·6	41·0	24·0 *
44·2	51·3	38·4	22·4 *
42·6	43·5	34·9	23·8 *
39·4	151·0	13·3	19·6 *
40·4	107·4	15·7	23·6 *
39·9	84·7	15·5	17·6 *
39·9	69·6	18·6	18·0 *
40·2	59·2	20·5	19·4 *
40·3	50·0	21·6	17·8
38·3	42·5	23·4	18·0 *
41·1	52·7	20·3	21·0 *
39·6	39·7	39·7	22·6 *
38·3	40·7	29·5	20·0 *

The linear part of the $\dot{V}$-pCO_2 relation at constant pO_2 may be expressed by the equation

$$\dot{V} = S(pCO_2 - B) \;.\quad .\quad .\quad .\quad .\quad .\quad .\quad (2)$$

where S is the slope and B is the intercept on the pCO_2 axis. This form is convenient for examining the effects of pO_2. The estimates of S and B obtained from a given set of data depend on the statistical treatment used; as we have been interested more in describing the relation between observed $\dot{V}$ and observed pCO_2 than in predicting $\dot{V}$ from pCO_2, we have used the reduced major axis, which tends to be close to the "best" line drawn by eye, rather than the more commonly used regression lines. The values for B and S given in Table II represent the reduced major axis for each pO_2 in each experiment.

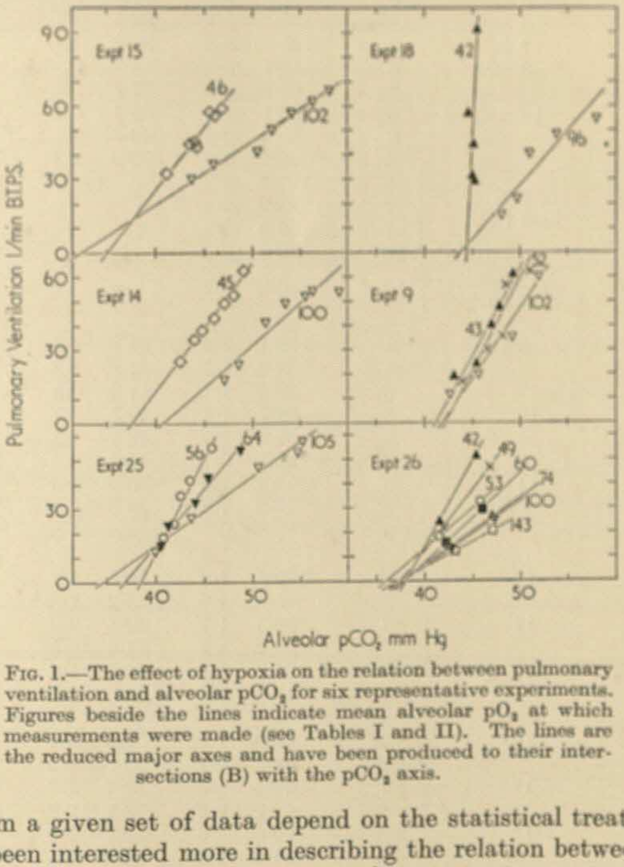

FIG. 1.—The effect of hypoxia on the relation between pulmonary ventilation and alveolar pCO_2 for six representative experiments. Figures beside the lines indicate mean alveolar pO_2 at which measurements were made (see Tables I and II). The lines are the reduced major axes and have been produced to their intersections (B) with the pCO_2 axis.

Two or three pO_2's were usually employed in the earlier experiments, which were mainly concerned with the linearity of the $\dot{V}$-pCO_2 relation and with the effect of pO_2 on B. In experiments 26 and 27 seven and eight pO_2's respectively were used, so that the effect of pO_2 on S could be examined; the results on subject 4 were also used for this purpose, values of S being calculated from the pooled observations at each pO_2 of $\dot{V}$ and of pCO_2 minus the value of B appropriate to the day.

TABLE II.—STATISTICS OF REDUCED MAJOR AXES FOR $\dot{V}$ AND ALVEOLAR pCO_2 AT VARIOUS ALVEOLAR pO_2'S

Subject	Experiment	No. of points	Alveolar pO_2	±s.d.	B	±s.e.	S	±s.e.
4	4	15	155·9	6·8	36·3	0·9	2·8	0·2
	5	6	155·8	3·9	37·6	1·1	2·8	0·3
	6	10	152·5	4·1	38·8	0·7	4·0	0·3
	7	7	155·0	6·3	36·8	0·8	3·0	0·2
	8	5	156·1	6·6	34·7	1·5	2·9	0·4
		5	64·9	2·5	37·7	1·0	4·8	0·6
	12	5	100·1	1·4	38·7	1·0	4·9	0·6
		5	49·8	2·5	40·6	0·8	9·9	1·8
		4	40·7	1·8	39·6	1·3	13·4	6·6
	5, 7, 8 and 12	8	101·0	1·7	..	..	3·3	0·7
		7	83·9	2·1	..	..	4·4	0·7
		7	64·5	2·1	..	..	4·6	0·5
		10	48·6	2·4	..	..	6·6	1·4
	4, 5, 6, 7 and 8.	43	155·0	5·7	..	..	2·8	0·1
20	9	4	102·1	2·5	41·8	1·6	5·4	1·2
		5	52·0	1·9	42·2	1·3	7·5	1·6
		5	42·6	1·2	41·2	0·8	7·4	1·1
	10	5	98·7	1·1	40·3	2·7	4·9	1·4
		6	49·6	1·9	42·2	1·3	9·2	2·2
		5	41·7	0·7	44·3	0·9	19·2	8·1
	11	5	97·3	1·5	36·0	3·0	3·3	0·8
		6	48·2	1·5	38·6	2·4	4·9	1·5
		5	40·6	0·7	40·5	1·8	8·8	3·1
21	13	7	100·4	1·6	40·8	1·5	3·2	0·4
		7	45·6	1·0	39·2	1·3	6·8	1·2
	14	7	99·3	1·4	40·7	2·0	3·4	0·5
		7	45·0	1·2	37·6	0·4	5·3	0·2
	15	7	101·7	1·4	32·5	1·5	2·6	0·2
		7	45·6	0·6	35·0	0·9	5·2	0·5
22	16	7	98·9	1·6	39·0	3·0	2·8	0·6
		6	44·2	0·7	39·8	1·8	8·3	2·8
	17	7	99·4	1·3	39·7	1·3	2·6	0·2
		8	42·9	0·9	38·7	2·2	3·7	0·8
	18	5	95·8	2·0	43·7	2·0	4·3	0·9
		5	41·6	1·3	44·5	0·7	84·8	46·8
23	19	8	100·2	1·4	41·2	2·8	4·1	0·9
		7	43·3	1·1	42·8	0·8	10·3	2·2
	20	7	101·0	0·8	39·3	2·0	3·1	0·4
		6	41·3	0·6	42·2	1·1	6·8	1·0
24	21	7	102·0	1·4	42·1	0·8	4·2	0·3
		6	45·4	1·5	40·0	1·3	7·6	1·5
	22	7	101·9	1·3	41·5	0·7	4·1	0·2
		7	45·6	0·8	41·2	1·1	10·9	2·5
25	23	4	94·2	6·1	40·6	2·1	4·7	1·0
		5	45·4	0·8	41·3	1·5	8·2	2·6
7	24	5	107·2	1·3	32·4	2·0	2·9	0·4
		4	54·4	0·6	35·6	0·9	5·6	0·6

TABLE II.—(*continued*)

Subject	Experiment	No. of points	Alveolar pO_2	±s.d.	B	±s.e.	S	±s.e.
7	25	5	105·4	1·5	34·7	1·0	2·8	0·2
		5	64·0	2·2	36·8	0·7	4·7	0·4
		5	55·9	1·6	38·4	0·5	7·9	0·8
	26	2	143·4		37·4		2·1	
		2	100·5		37·8		2·7	
		2	74·0		37·6		2·8	
		2	60·3		38·0		3·8	
		2	52·6		36·0		3·4	
		2	48·8		37·6		5·2	
		2	42·2		38·1		7·2	
	27	2	141·0		34·7		2·8	
		2	106·6		35·8		3·4	
		2	84·5		36·8		4·9	
		2	67·2		32·9		2·6	
		2	57·4		34·9		3·9	
		2	52·0		37·6		5·8	
		2	43·0		29·6		2·7	
		2	40·2		34·5		7·8	

DISCUSSION

It is generally agreed [Gray, 1950] that the relation between $\dot{V}$ and pCO_2 is linear over the middle range, which we have investigated (pCO_2 not less than *ca* 1 mm. above resting; $\dot{V}$, with one exception, not more than 80 l./min.). Scatter diagrams of our results (*e.g.*, fig. 1) bear this out, and statistical tests for non-linearity have not been made.

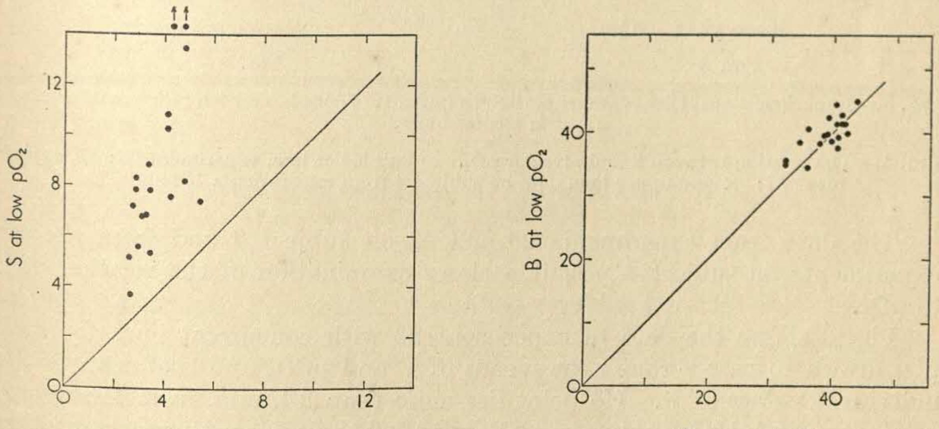

FIG. 2.—The different effects of hypoxia on B and S.

Table II shows that B is effectively independent of pO_2, in contrast with S, which varies inversely with pO_2, and these conclusions are illustrated in fig. 2. The points obtained by plotting B at low pO_2 against B at normal

pO_2 for each experiment lie close to the line of equality. The absence of material change in B as pO_2 changes implies that the $\dot{V}$-pCO_2 lines at different pO_2's are concurrent on the pCO_2 axis. This has been tested (on the suggestion of Dr. R. B. Fisher) by showing that concurrent regression lines (of pCO_2 on $\dot{V}$) fit these data as closely as do regression lines calculated independently for each pO_2.

For S, on the other hand, all the points in fig. 2 lie to one side of the line of equality. In general, S increases, sometimes very greatly, as pO_2 decreases. Gray [1950] stated that pO_2 affected B, and had no effect on S. These assertions are refuted by the present data, which are fully consistent with those of Nielsen and Smith [1951] and of Cormack *et al.* [1957].

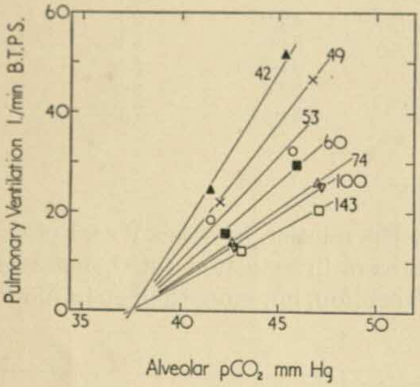

FIG. 3

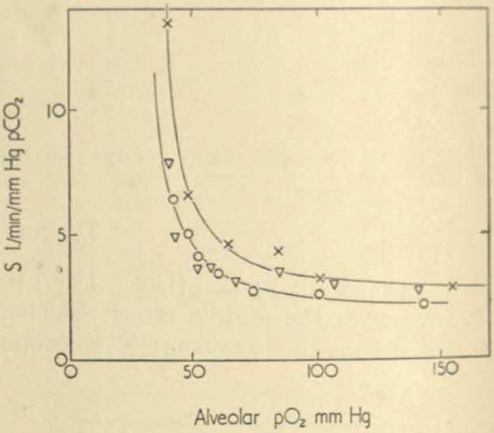

FIG. 4

FIG. 3.—Experiment 26. Observations plotted as in fig. 1. Lines have been redrawn concurrent on a common B.

FIG. 4.—The relation between S and alveolar pO_2. ○ and lower line, experiment 26; ▽, experiment 27; × and upper line, data on subject 4 from experiments 4–8 and 12.

The data from experiments 26 and 27 on subject 7 and from the six experiments on subject 4 permit a closer examination of the relation of S to pO_2.

Fig. 3 shows the data of experiment 26 with concurrent lines for each pO_2 drawn to pass through the means of $\dot{V}$ and pCO_2 and the mean of the individual values of B. No point lies more than 2 l./min. from the appropriate line, and the fan of lines may be regarded as an adequate representation of the data. S read from fig. 3 is plotted against pO_2 in fig. 4, along with similarly determined values from experiment 27 on the same subject, and with values from the pooled data on subject 4.

The upper curve in fig. 4 has been fitted by the method outlined below to the data from subject 4 and the lower curve to the data from experiment 26. The curves show that as pO_2 rises to high levels, S falls towards an asymptote (the value when no hypoxia is present), and that it rises to very high values as pO_2 falls.

There are many expressions which can give curves of this type. Two, among others, have been considered by us:

$$S = D\left(1 + \frac{A}{pO_2 - C}\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and

$$S = E.\,10^{\frac{H}{pO_2 - G}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

With both expressions S falls to a limit (D or E) as pO_2 rises, and rises to high values as pO_2 falls to a limit (C or G).

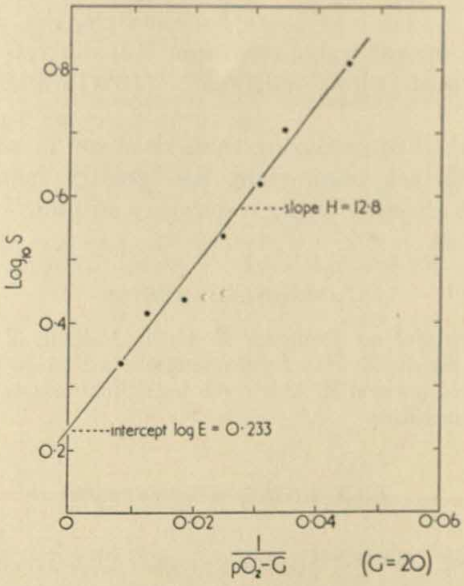

FIG. 5.—Estimation of the parameters of experiment 26. The line has been drawn by eye.

The parameters in equation 4 may be estimated from a set of data by assuming a likely value of E and plotting $\frac{1}{\log S/E}$ against pO_2 to get a value for G, and then plotting log S against $\frac{1}{pO_2 - G}$. The iteration is continued until final values of E and G, and hence H, are obtained. The second plot for experiment 26 is shown in fig. 5.

The parameter B is a little less than the resting pCO_2, and it seems from Nielsen and Smith's work that below B a change in pCO_2 does not affect $\dot{V}$. We have no data for these levels. Like the resting pCO_2, B appears to be affected by ingestion of ammonium chloride or sodium bicarbonate [Nielsen, 1936] by exercise [Bannister, Cunningham and Douglas, 1954; *cf.* Asmussen and Nielsen, 1957] and by acclimatization to high altitudes. Cunningham and O'Riordan [1957] found no evidence that it is affected by temperature.

E (or D) is the slope of the $\dot{V}$-pCO_2 line when no hypoxic stimulus is present. Our data imply that the slope under normal pO_2 is appreciably greater than E; *i.e.*, there is a hypoxic stimulus affecting ventilation when air is breathed at sea-level. This supports the conclusions of Watt, Dumke and Comroe [1943] of Loeschcke [1953] and of Dejours, Labrousse, Raynaud and Teillac [1957]. The data of Lambertsen, Ewing, Kough, Gould and Stroud [1955] provide some support for our view that E (or D) has a finite value, *i.e.*, that CO_2-sensitivity does not tend to zero as pO_2 tends to infinity.

The physiological significance of H (or A) is obscure, and that of G (or C) is little clearer. There is no evidence, for example, that it is closely related to the critical value for pO_2 (about 30 mm. Hg) derived by Cormack *et al.* [1957] from the data of Dripps and Comroe [1947] obtained on hypocapnic subjects.

The present method of analyzing these data on $\dot{V}$, pCO_2 and pO_2, with the use of four distinct parameters, has greatly facilitated subsequent investigations of the effects of other respiratory stimuli.

ACKNOWLEDGMENTS

M. G. M. J. is grateful to Professor E. G. T. Liddell, F.R.S., for laboratory facilities. We thank Mr. E. N. Hey for drawing our attention to the reduced major axis, Messrs. T. J. Meadows and E. Aldsworth for skilled assistance, and the subjects for their cheerful participation.

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BOOK REVIEWS

BIOCHEMICAL DISORDERS IN HUMAN DISEASE. Edited by R. H. S. Thompson and E. J. King. London: J. & A. Churchill. 1957. Pp. xiv + 843. £4, 10s.

There can be no question that this will prove a most valuable publication and the editors and contributors deserve high praise for their initiative and industry in ministering to its creation. With the present-day orientation of medical research the scope of the book's title is nearly co-extensive with that of medicine so far as recent additions to knowledge are concerned, and this submission is upheld both by the table of contents and by the actual text. Since each of the twenty chapters is of different authorship, brief comments will be offered upon each individually, and these will be followed by a few general observations regarding the book as a whole.

The opening chapter by D. A. K. Black is devoted to disorders of the gastro-intestinal tract; it necessarily embraces a wide variety of pathological conditions but a biochemical emphasis is kept well to the fore, and especially in the sections on electrolytes and sprue the author can draw extensively on his own observations. Chapter Two is on the liver and is written by N. F. Maclagan, who combines a general account of the pathological physiology of that organ with some very practical guidance on diagnostic problems. The anæmias are dealt with by J. R. P. O'Brien and S. T. Callender who provide a very comprehensive, if slightly non-committal, review of the many biochemical considerations bearing upon the health or otherwise of the erythron. A much more individual approach is adopted by R. G. Macfarlane in his essay on the clotting mechanism, in which an impressive degree of coherence is attained without disguising the complexities and obscurities of the subject. In their account of hypertension, G. W. Pickering and W. S. Peart summarize information on the nature and incidence of this condition and on the biochemistry of pressor agents which may be implicated in its production. M. D. Milne's chapter on the genito-urinary system, including the kidney, is a straightforward and up-to-date reprise of this traditional theme in clinical chemistry. J. W. Jailer and D. Longson follow with a sensible and clear exposition of current ideas on function and dysfunction in the adrenal cortex and medulla. The endocrine glands also figure in the two succeeding chapters of which the first, headed—with a hint of preciosity—"disorders of iodine metabolism", is by J. Wolff and R. C. Goldberg. It contains the word "thyroid" in the first and last sentences and is almost wholly devoted to that gland. The investigation of thyroid function, including tests with radio-iodine, and the pharmacology of antithyroid drugs are well handled but the descriptions of hyper- and hypothyroidism go rather beyond what is required as a background for expounding their chemical pathology. The remaining chapter of this group is by Russell Fraser and E. J. King; it gives an excellent survey of modern ideas on bone-mineral and on the process of mineralization, and also covers calcium and phosphate metabolism generally, including the parathyroid gland. Editorial representation is continued in the chapter on the nervous system by R. H. S. Thompson and J. N. Cumings. Here a neat blend of the dynamic and analytical streams in biochemistry is achieved and forcefully illustrates the progression of neurology out of its rather arid anatomical past. The chapter on diseases of muscle by the late J. Lilienthal and K. L. Zierler proceeds systematically from physiology to pathology, the physiological section being particularly well presented. The chapter on diabetes by P. H. Forsham and G. E. Mortimore, though very informative, is not entirely satisfactory; it is packed with erudition but is overschematized and disjointed. R. Passmore and A. P. Meiklejohn, in their chapter on nutritional disorders, boldly place atherosclerosis into this category; their discussion of obesity and malnutrition is notable for sound common-sense and their account of vitamin-deficiencies has the ring of first-hand experience. H. Harris contributes a useful account of the disorders of amino-acid metabolism which have been recently elucidated and of their genetic relationships. E. G. L. Bywaters and L. E. Glynn have a chapter on connective tissue disorders which covers in a discursive fashion efforts to apply biochemical and biophysical methods to the study of these disorders and includes a useful section on gout. Short serviceable chapters on the porphyrias by C. H. Gray, on hæmochromatosis by S. Granick, on glycogen disease and galastosæmia by D. H. Anderson and on the lipidoses by S. J. Thannhauser lead up to the final chapter of the book which is by P. M. F. Bishop and I. F. Sommerville. This has as its subject reproductive disorders and Dr. Bishop's section is notable for an admirably concise summary of the findings, so far as hormone assays are concerned, in some sixteen types of disturbance affecting the generative organs. Dr. Sommerville discusses the technical aspect of hormone assays.

The editors mention in their preface that they have deliberately chosen to arrange their material on an "organ basis" but the ideality of a classification which results in no less than six out of twenty chapters being headed "miscellaneous disorders of metabolism" can hardly go unquestioned. For so composite a work instances of overlap are remarkably few, although the metabolism of hæmoglobin does seem to crop up somewhat repetitively in a number of places. The selection of topics for inclusion in such a work is bound to be arbitrary but a brief section on what is known and thought of the biochemical effects of ionizing radiation might perhaps have reasonably claimed a place. However, the main impression by this book is overwhelmingly favourable, for it may be said to supply at once an eminently readable survey of many advancing fronts in medical knowledge and also a most valuable compendium for reference purposes. The book is printed on rather bulky paper but the printing and illustrations are very clear.

R. V. Coxon.

HISTOLOGY. By A. W. HAM. London: Pitman Medical Publishing Co. Ltd. Pp. xv + 894 with 582 Illustrations. 3rd Edition. 1957. 80s.

In the first two editions of this book, Dr. Ham established relationships between histology and many other disciplines. These correlations have now been augmented by the results of high resolution electron microscopy, so it is logical that in this new edition these recent advances should play a large part. Some new textbooks have ignored these advances and others have included micrographs but have not modified the text. This edition, however, can be considered a new book, since the relationship between macromolecules, fine structure and light microscopy are stressed throughout the text. Apart from the general argument that structural concepts should not be limited by the technique which happens to be convenient to elucidate them, Dr. Ham argues that electron micrographs should be included to supplement a student's practical light microscopy. With good reproductions, a textbook like this allows the student to study the material on which the electron microscopist made his observations, for it is the micrograph which is the counterpart of the microscope slide.

The junior student is still catered for in spite of the inclusion of so much new material. Although there is a small increase in actual size, over seventy new illustrations mean that the text has been relatively reduced. This limitation has been achieved by the elimination of controversies which are now obsolete, and by some condensation of the verbose but readable style of the earlier editions.

The statement in the Preface that there has been a revolution in histology should be put in the present tense, for even since this book was published there have been many reports which would modify the existing text, notably Weiss on the splenic sinusoids, Huxley on striated muscle and Uzman and Moran on peripheral nerves. Such modifications can be incorporated in later editions without detriment to the existing form of the book. All students of histology should see this book, but it should also be consulted by anyone whose researches are even indirectly based on concepts of tissue structure.

A. R. MUIR.

MORPHOLOGIE UND PHYSIOLOGIE DES NERVENSYSTEMS. Von Prof. Dr. PAUL GLEES, Oxford. Stuttgart: Georg Thieme. 1957. Pp. xi + 445 and 149 figs. D.M. 58.

The title—morphology and physiology of the nervous system—might apply to encyclopædic surveys, textbooks or books on selected topics. Dr. Glees' book, with 445 pages which comprise 149 large and beautiful figures, can obviously only belong to the last category. Anatomically speaking, most parts of the nervous system are dealt with. The aspects touched upon a range from electron microscopy and histology to biochemistry, physiology, neuropharmacology and psychology. In writing such a book, the greatest problem must have been the selection of the most important and most reliable material from a wealth of literature which no single individual can be really familiar with, let alone evaluate critically.

This volume is not a textbook; it is addressed to the young clinician or to the physiologist who is not specialized in neurology but wishes to get acquainted with modern trends and methods of neurological research. Reading about unsolved problems should stimulate him to look up the original literature and challenge him to attack some of the questions on his own. The references, many of which are from the German literature and therefore less familiar to the British reader, are one of the most essential features in such a work. As is inevitable, much of the text is based on review articles; but it is in the often haphazard selection of the references and in the resulting arbitrariness with which certain problems are put that fault will be found with the book. To give just one example. In the references to the chapter on acetylcholine as transmitter at the motor and plate there is no reference to H. H. Dale (except for a paper written in 1914) or to G. L. Brown; this is strange even for an author who professes not to be interested in humoral transmission. It is to be hoped that, when the book sees further editions, much of the sketchiness of the presentation will disappear.

Marthe Vogt.

DIE PHYSIOLOGIE DES LYMPHOZYTENWECHSELS UND SEINE BEEINFLUSSBARKEIT DURCH HORMONE DES HYPOPHYSEN-ADRENALSYSTEMS.

By Priv.-Doz. Dr. H. G. Hansen. Stuttgart: Georg Thieme. 1958. Pp. vii + 164 and 74 figs. D.M. 18.50.

This work is essentially a monograph on the fate of the lymphocyte in the mammalian organism. The experimental work was carried out on the cat. The introductory chapters deal with the anatomy and histology of the lymphatic system, with surgical procedures suited to obtain lymph from the cat's thoracic duct, and with rate of flow and number of lymphocytes obtained in experiments on anæsthetized cats over prolonged periods of time. The author was able to demonstrate that the lymphocytes entering the blood from the thoracic duct are the main source of these cells in the circulating blood. By labelling the lymphocytes with ^{32}P or with fluorescent dyes, the sites invaded by the lymphocytes were rendered visible. The author estimated the survival time of the cells in the circulating blood to be no more than 18 hr. The further fate in the tissue could not be followed with certainty, but the fact that lymph glands are an effective trap for lymphocytes precludes the possibility of re-circulation of a cell which has left the blood stream. Lymphocytes which are labelled *in vitro* and then re-infused are very rapidly trapped by the lungs and then probably destroyed by liver and spleen.

The part of the book concerned with the effect of ACTH and cortisone or cortisol is of limited interest, because effects were only obtained with excessively high doses, up to 200 mg. cortisol per 24 hr. These doses depress lymphopoiesis. Some evidence of increased trapping and destruction of lymphocytes in the tissues was also obtained. The failure to observe lymphopenia with smaller doses of corticoids might have been due to the fact that the experimental conditions by themselves produced hyperactivity of the adrenal cortex and therefore a base-line which was little suited to show the effects of further increases in adrenocortical hormones.

A critical evaluation of the clinical usefulness of therapy with cortisone or ACTH in childhood concludes the book. Any reader interested in the physiology of the lymphatic system will find the experimental part fascinating and full of new facts about the dynamic state of the lymphatic tissue. The book will, however, be of equal interest to the hæmatologist and the clinician interested in diseases of the lymphatic system.

MARTHE VOGT.

THE RESPIRATORY MUSCLES AND THE MECHANICS OF BREATHING.

By E. J. M. CAMPBELL. London: Lloyd-Luke. 1957. Pp. xvi + 131. £1.

Accounts of the actions of the muscles of respiration have found their way almost unaltered from one textbook to another for the last thirty years. Dr. Campbell reviews the whole subject and reports his own electromyographic studies; in doing so he illuminates our ignorance and gives us much food for further thought.

An account of the known facts concerning the respiratory actions of individual muscles occupies half the book and will provide a standard reference source. Other chapters deal with the work of breathing, the pressure-volume diagram, the clinical examination of the respiratory muscles and relevant aspects of electromyographic technique. It is in the sections on the nervous control of respiration and the restraints upon maximum respiratory effort that the author becomes most stimulating —and, it must be said, controversial. Current concepts derived from animal experiments postulate the dominance in man of a background of inspiratory tone upon which inhibitory mechanisms operate. Dr. Campbell's case against this seems to the reviewer unproven. Nor can the absence of electromyographic evidence of expiratory muscle activity during either quiet or considerably augmented breathing—even if this is taken to exclude such activity—be adduced as evidence against the participation of an expiratory centre in human respiration; this might act solely by inhibiting inspiration. Nevertheless, Dr. Campbell convincingly calls into question the applicability to man of certain of the results of animal work.

This is not an easy book to read. It is, however, a valuable addition to the literature in a neglected field and is destined to be much thumbed by the curious in both physiology and clinical medicine.

E. A. HARRIS.

BOOK NOTICES

Ergonomics. Human Factors in Work, Machine Control and Equipment Design. General Editor: A. T. Welford. London: Taylor & Francis Ltd. Volume 1, Number 1, November 1957. Pp. 100. £1, 5s. The Ergonomics Society has now an official quarterly publication with an impressively international editorial board. The sub-title is "Human Factors in Work, Machine Control and Equipment Design", and the first number contains eight papers and two summaries of papers published elsewhere, together with a list of members and the current proceedings of the Society. The Editorial expresses the need which it is hoped to fill: "Both research workers and industry have been aware of difficulties on the one hand, of making research understandable to 'users' and on the other, of bringing field problems to the attention of those interested in studying them."

While industrialists are thus encouraged to provide discussion of research work and to give accounts of the work and organization of their departments, the majority of contributors to the first number appear to be full-time research workers. At the same time, however, considerable care has been taken to ensure that there is something for each group of readers which it is hoped to attract. The consequent variation in approach and level of the articles may make the journal less acceptable to those with closely defined interests. Nevertheless there seems a genuine need for just such a publication, and it should prove of value to physiological departments which have some association with industrial problems.

The Autonomic Nervous System. *Brit. Med. Bull.* **13**, 153–230. 1957. £1. Edited By W. Feldberg. In a masterly introduction Sir Henry Dale says that he had expected that some at least of the contributors would not feel limited by the anatomical title of the volume. Most of the articles do, however, deal either with the autonomic nervous system or with the chemical transmission of nervous impulses elsewhere. There are articles on the metabolism of the substance liberated at nerve endings [Birks and McIntosh; Blaschko and Vogt] on their mode of action on the heart and smooth muscle [Bülbring, Hutter and Burn], on sensory receptors [Gray and Diamond], vasodilatation in salivary glands [Hilton and Lewis] sweat glands [Lovatt Evans], anatomical relationships [Garry, Boyd, Murray and Thompson] and on transmission in autonomic ganglia [Perry]. This volume is a most valuable summary of recent work in this field.

The Neurohypophysis. Edited by H. Heller. Proceedings of the Eighth Symposium of the Colston Research Society held in the University of Bristol. London: Butterworth Scientific Publications. 1957. Pp. xv + 275. 50s. The published proceedings of scientific conferences often make dull and unsuccessful books. They are not written to appeal to readers but rather to set down for posterity a mass of material drawn together for the conference. The present book, however, makes agreeable reading throughout, possibly because it is the fruits of the first international conference devoted solely to this important field. Consequently more attention has been given to the development of each aspect, which from the general readers' point of view is most helpful.

There are eighteen lectures covering the historical, anatomical, physiological and biochemical aspects of the neurohypophysis. Among many other matters Sir Henry Dale reminds us "So far as I am aware, there is no other instance yet known in the endocrine system of higher vertebrates in which not only the output of a hormone from an endocrine gland but its production and maintenance there, are under such complete and immediate nervous control, as seems undoubtedly to exist in this case. The severance of the nervous connexion between the neural lobe and the hypothalamic nuclei leads to the disappearance of the hormones from the lobe within a few days." It is not surprising, therefore, to find that this symposium has a considerable physiological bias. Indeed some of the most important evidence concerning the way in which the endocrine activity of the neurohypophysis is naturally excited and controlled has come from perhaps physiology's most elegant preparation—the normal conscious trained animal. Such preparations coupled with the very careful biochemical work on the nature of the hormones have resulted in considerable advances in our knowledge.

As befits a book of permanent value it is well printed on high-grade paper and the production is excellent.

REGULATIONS AND MODE OF ACTION OF THYROID HORMONES. Ciba Foundation Colloquia on Endocrinology. Vol. 10. London: J. & A. Churchill, Ltd. 1957. Pp. xii + 311. 48s. These eighteen lectures were originally delivered under the chairmanship of Dr. Rosalind Pitt Rivers from whose pioneer work on the iodinated thyronines much of the content of this symposium has stemmed. The lectures deal with the control of the thyroid gland, the nature of the thyroid hormone or hormones and the mode of action of these or their derivatives at the cellular level. In each of these aspects many puzzling new facts have arisen in the last decade. The old notion of a simple mutual inter-relationship between the anterior lobe of the pituitary and the thyroid is no longer tenable, and indeed the complexity of new ideas is such that it is now far from clear how the thyroid gland is controlled. It was hoped that, as the latent period of action of triiodothyronine or triiodothyroacetic acid was less than that of thyroxin, these substances might prove to be the active compounds at the cellular level. Work reported in this volume has not substantiated these early hopes. With the field ranging from the bedside to the chromatographic cabinet it is inevitable that few will be interested in the whole book; but all concerned with the thyroid gland will find less familiar aspects well documented. The whole subject of the thyroid gland is now complex and controversial and some of the most interesting parts of the book are the records of the vigorous discussion after each paper. The Chairman summed up the conference with these words: "It has certainly provided much food for thought and although I feel that I am not much nearer knowing what the thyroid hormone is or does, at least it has given us some stimulus towards new experiments".

DIGITALIS. Edited by E. Grey Dimond. Springfield: Thomas. Pp. xiv + 255. 52s. 6d. In February 1956 the University of Kansas held a symposium on digitalis covering pharmacological and clinical aspects. The proceedings are published in this book. The sections dealing with the metabolism and excretion of the drug and with the relationship of potassium to its action are of particular interest. Much of this material is new. Apart from an important chapter on digitalis-induced arrhythmias the clinical sections follow mostly conventional lines.

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AN EXPERIMENTAL NUTRITIONAL MYOPATHY AND NEUROPATHY.

By J. B. CAVANAGH and I. MACDONALD. From the Departments of Pathology and Physiology, Guy's Hospital Medical School, London, S.E.1.

(*Received for publication* 11*th December* 1957)

Adult rabbits fed on a diet largely composed of casein and sucrose develop a myopathy and occasional degenerative nervous changes the severity of which may be related to the length of time on the diet. In one long surviving animal widespread sensory neuropathy developed.

Parenteral α-tocopherol acetate failed to prevent the nerve and muscle changes.

IN the course of feeding experiments on rabbits carried out by one of us, about 25 per cent of the animals developed severe flaccid weakness of the limbs accompanied by marked muscular wasting. One animal (118) showed these signs very markedly and there were acute degenerative changes in certain of the sensory tracts of the spinal cord and nerve roots as well as in the peripheral nerves and muscles. The precise alterations in this animal are detailed below, but because of the selectiveness of the nervous lesions, their similarity to certain human conditions, especially those associated with carcinoma, and the fact that such changes have not been produced experimentally in the rabbit before, it was decided to study a further series of animals. Moreover, because α-tocopherol had been omitted from the diet in the first experiments and its deficiency has been reported as causing myopathies in rabbits [Pappenheimer, 1943] half the animals in the present series were given parenteral injections of this vitamin. Although the results, so far as the further reproduction of the sensory neuropathy is concerned, have been disappointing, they are not without interest in connection with the recognized but obscure relationship of vitamin E to neuromuscular defects.

METHODS

Twenty adult rabbits were given a diet of 35·5 per cent calcium caseinate, 57·5 per cent sucrose, 2 per cent yeast and 5 per cent salts with added vitamins A and D [Macdonald and Thomas, 1956]. Ten of these animals (Group B; the remainder will be referred to as Group A) received in addition twice weekly intramuscular injections of 3 mg./kg. body weight α-tocopherol acetate. Three animals in each group were not examined histologically, as they were found dead and were thus of very limited value. Also included in the data to be discussed are rabbits not members of this special series, but which had been kept on the same diet and which showed clinical evidence of muscular weakness and "paresis" but in which no attempt was made to record the histological changes.

An inspection for any evidence of limb weakness was made at the time of daily weighing. When severe weakness made the likelihood of further survival small, the animal was killed with an intravenous overdose of nembutal, and the body dissected immediately. The skinned limbs were fixed in formal-saline *in toto* to prevent distortion of the muscles and nerves; the spinal canal and brain case were opened to allow the rapid penetration of the formal-saline and the neuraxial tissues removed when sufficiently hardened. Blocks of tissue were subsequently embedded in paraffin wax and sections stained with hæmatoxylin and eosin: other special stains such as the Swank-Davenport method, Glees and Marsland's method for axons and myelin sheath stains were used when considered helpful.

RESULTS

Functional Observations.—Severe paralytic weakness was encountered in only two out of the twenty animals in the series (196 and 201, figs. 1 and 2) and also in the original animal 118; a lesser degree of weakness was present in series animal 198. Previously seventeen out of sixty-nine rabbits given the same diet had been found to have limb weakness but at this period no histological studies had been undertaken to ascertain whether structural elements were affected. The "paresis" which was apparently of the same nature and again encountered in the new series, consisted of lack of co-ordination particularly noticeable in the hind limbs and an easily evoked state of muscular fatigue, superficially resembling myasthenia gravis. In animal 118 it reached its maximum development, the animal being almost unable to move, except for movements of its jaws and head and some ill-sustained struggling. The limbs were hypotonic and flail-like; wasting of muscles was severe; the tendon reflexes were absent; responses to pin-prick and tendon-squeezing below the knee and elbow were negligible. Particular interest was paid to response to pin-prick in other weakened animals as this was the only possible guide as to whether neuropathy of the sensory apparatus was present or not. All weakened animals, however, appeared to have a "glove and stocking" analgesia, but only in animal 118 was this finding correlated with definite evidence of nerve and tract degeneration. In animals 196 and 201 severely weakened as noted above, a similar "glove and stocking" analgesia was found.

The length of time on the diet, together with a record of the presence or absence of neurological signs in the control and test groups of animals are seen in Table I. It can be seen that no significant difference exists between the group of animals receiving α-tocopherol and those not receiving it, except in

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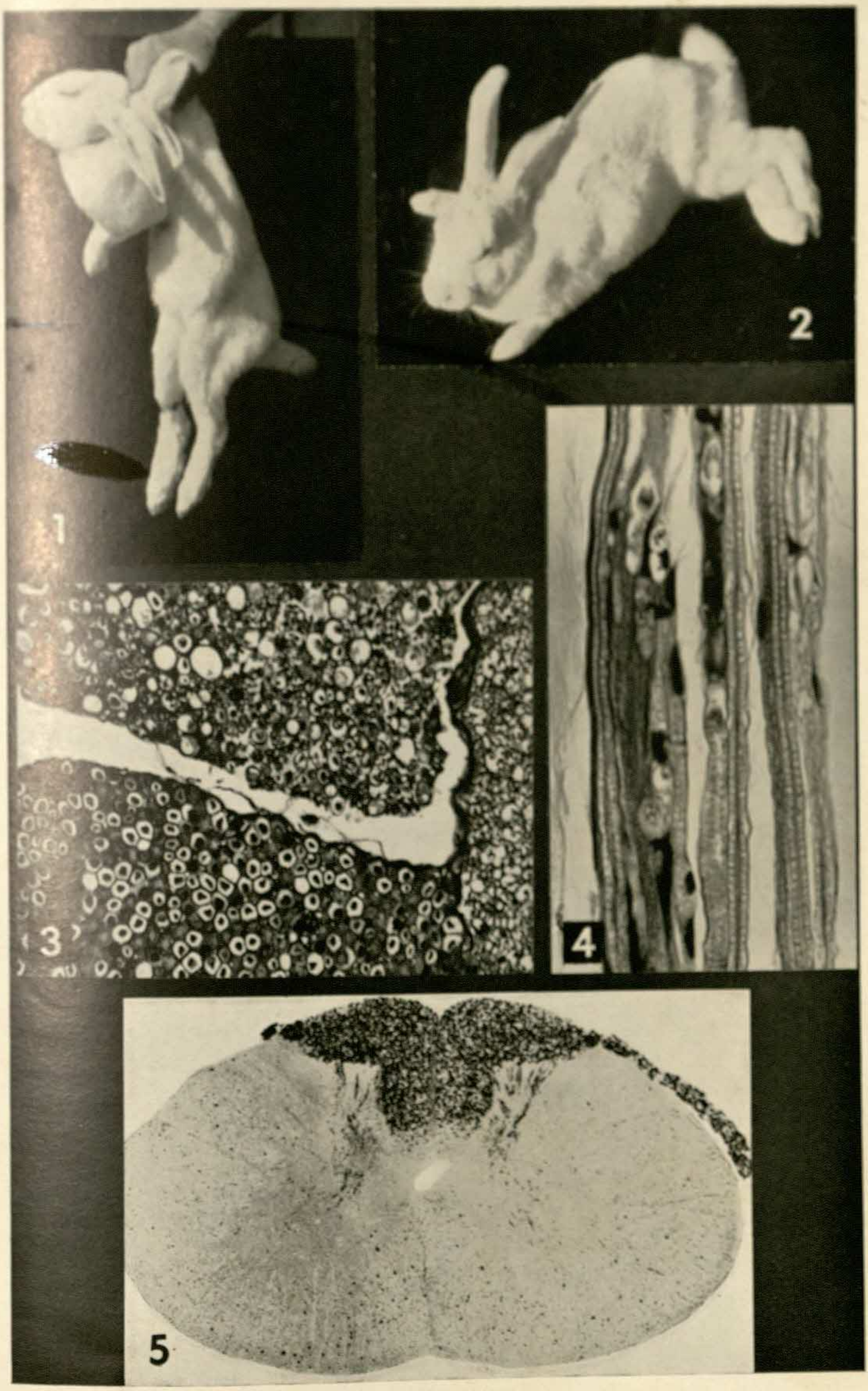
FIG. 1.—Animal 196 suspended to show the wasted hind limbs held slightly in adduction.

FIG. 2.—Animal 201 lying on the floor showing wasting and abnormal posture of the hind limbs and splaying of the fore limbs.

FIG. 3.—Animal 118. Dorsal root above is severely damaged; the ventral root below is normal. Lumbar level. × 180. Hæmatoxylin and eosin.

FIG. 4.—Animal 118. Sciatic nerve showing both normal fibres and Wallerian changes in other fibres. × 535. Hæmatoxylin and eosin.

FIG. 5.—Animal 118. Severe degeneration confined to the dorsal columns and the fibres ending in the dorsal grey horns. Lumbar cord. × 15. Swank-Davenport.



FIGS. 1–5

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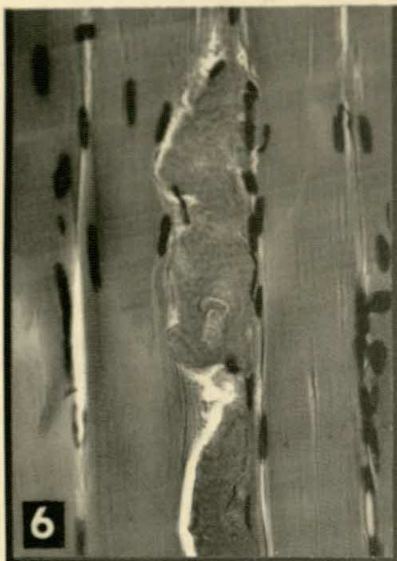
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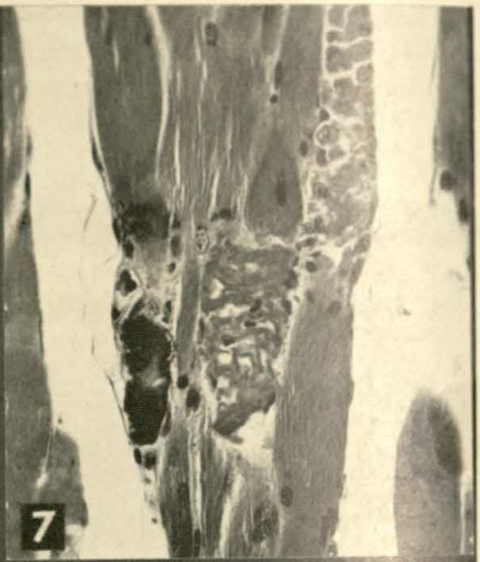
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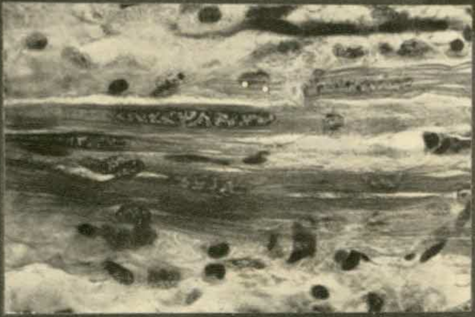
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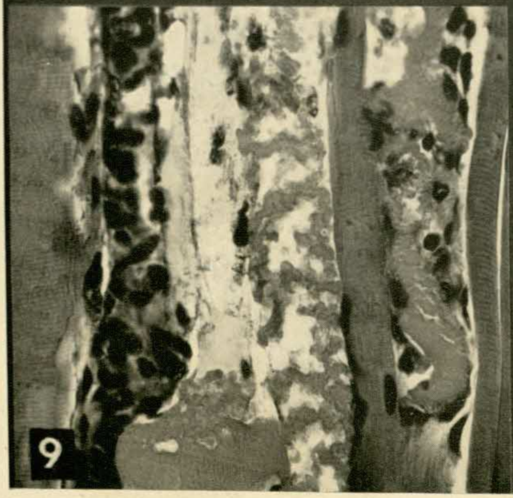
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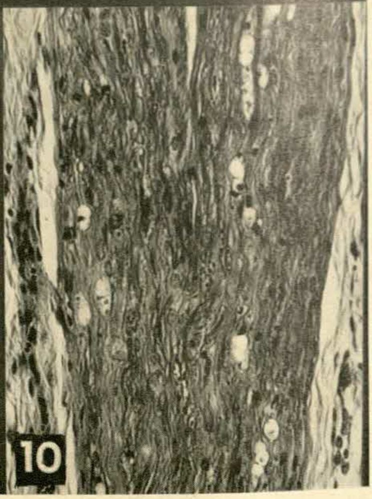
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FIGS. 6–10

the survival times. The mean survival time was 75 days for those on the diet alone and 56 days for those having the intramuscular injection of α-tocopherol acetate and the diet. Statistical analysis showed that there is

TABLE I.—DATA OF THE RABBITS IN THE SERIES ON THE DIET WITH AND WITHOUT α-TOCOPHEROL ACETATE INJECTIONS

Animal number	Time on diet (days)	Percentage fall in initial body weight	Skeletal muscle changes		Nervous system changes	Remarks
			Recent	Late		
118	146	30·0	+ +	+	+ +	Severe "paresis"
196	46	22·4	+	+	0	"Paresis"
199	52	21·3	+	+	0	. .
195	58	26·0	0	0	+	. .
200	60	37·2	0	+	+	. .
193	65	31·3	+ +	+	0	. .
201	66	30·6	+ +	0	0	"Paresis"
192	81	13·3	+ + +	+ +	+	Respiratory infection
194	92	38·8	+ +	+	+	. .
198	104	23·5	+ + +	+ +	+	"Glove and stocking" analgesia
197	124	28·0	–	–	–	Diarrhœa
Mean	75	27·2	–	–	–	. .
Animals not having α-tocopherol acetate intramuscularly (Group A)						
210	34	9·6	0	+	0	. .
204	36	13·2	+ +	+	0	. .
211	43	20·8	0	0	0	. .
209	48	11·0	–	–	–	Respiratory infection
205	51	31·3	0	0	0	. .
208	54	13·4	+ +	+	+	Respiratory infection
207	60	23·7	+ +	+	0	Respiratory infection
206	74	21·5	+	+	+	. .
203	77	22·6	–	–	–	. .
202	85	35·5	–	–	–	. .
Mean	56	20·3	–	–	–	. .
Animals having α-tocopherol acetate intramuscularly (Group B).						

a 1 in 10 to 20 probability of these values arising by chance. The injections, therefore, did not prolong life on the diet, but if anything the reverse.

Structural Changes.—Rabbit 118: extensive and recent degeneration of the myelin sheaths and axons in the dorsal columns of the spinal cord was

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FIG. 6.—Animal 204. Recent focal necrosis in quadriceps muscle. ×370. Hæmatoxylin and eosin.

FIG. 7.—Animal 108. Recent and slightly older lesions and an area of calcification in quadriceps muscle. ×275. Hæmatoxylin and eosin.

FIG. 8.—Animal 198. Active regeneration in the adductor muscle. ×370. Hæmatoxylin and eosin.

FIG. 9.—Animal 208. Recent necrotic changes in muscle fibres with early macrophage response. ×370. Hæmatoxylin and eosin.

FIG. 10.—Animal 198. Intramuscular nerve showing many fibres undergoing Wallerian degeneration. ×180. Hæmatoxylin and eosin.

the most conspicuous histological feature. The changes were maximal in the lumbar regions and less marked on ascending the cord. Chiefly it was the long ascending fibres of the posterior roots that were affected but, as shown in the Swank-Davenport preparations the short fibres ending in the dorsal grey columns were also clearly degenerating (fig. 3 and 5). Scattered loss of nerve cells with reactive proliferation of satellite cells was found in the dorsal root ganglia, but the numbers of damaged cells appeared to be considerably less than expected from the number of degenerated dorsal root fibres. In the sciatic nerves Wallerian degeneration was present in many scattered fibres (fig. 4). The state of degeneration in both the nerves and the spinal cord indicated that the lesions were about 1 week old.

In the muscles of this animal there were numerous recent focal necroses and many foci of macrophage activity and regeneration, both of various ages, scattered throughout the muscles without any orderly pattern. Intramuscular nerve bundles occasionally showed Wallerian changes.

Animals in Groups A and B.—In the remaining animals the histological changes (Table I) will only be dealt with in general terms. Although it was not possible to reproduce the extensive damage found in rabbit 118, slight changes of the same nature were encountered in four animals of the first group and two in the second.

In the *spinal cord* in two specimens a few degenerating fibres were discovered after careful searching; such fibres were recognized by severe vacuolation and the presence of a myeloclast in the lumen.

In the *sciatic nerves* Wallerian degeneration of minimal extent was found in six animals. A maximum of five such fibres in any longitudinal section of one nerve was discovered and in most instances only one or two were present. Again these changes were also undoubted owing to the clear vacuolation of the myelin and axon and the presence of reacting Schwann cells and macrophages in the individual tubes (fig. 10).

The muscles showed a greater degree of change but there was considerable variation in intensity from one muscle mass to another. The adductors, the quadriceps femoris, gastrocnemius, soleus, the triceps and the erector spinæ muscles were sampled (figs. 6, 7, 8 and 9).

The erector spinæ muscles were involved in every case examined and the suralis muscle was usually affected more than the gastrocnemius. In all involved muscles there was an excess of recently formed lesions over old ones. In two animals (200 and 210) the appearance of regenerating muscle fibres were the only changes found. In one animal of group A (195) and in two of Group B (211 and 205) the muscles appeared normal.

While the overall picture was the same in the two groups, slight but significant differences were encountered. In Group A there was a greater tendency to find lesions of longer standing as well as recent necroses. It is noteworthy that lesions of moderate to severe degrees do not appear in Group A until the 65th day and longer on the diet. Before that time three out of the four animals were almost, if not entirely, free from any change. In Group B only one animal (206) lived beyond the 65th day; all animals, however, showed abundant recent necroses, and one animal had many older changes. Thus there is a definite tendency in this group towards earlier muscle changes. There was no clear correlation between the extent of the nervous lesions and the severity of muscle damage; rabbit 118 with extensive neural involvement had no greater muscle damage than others without this feature. Focal necroses were encountered in three of fifteen livers examined, two of them in the animals having additional α-tocopherol. The high incidence of pulmonary infections (Table I) in this group may have been in part responsible for this feature.

Discussion

Various lesions produced in animals have apparently been cured by the administration of vitamin E and chief among the structures involved are the liver and muscles. The fact that vitamin E was given parenterally in these animals and had no recognizable effect in preventing the development of lesions and may indeed have hastened their onset, leads us to suspect that other factors were responsible.

The absence of fat, apart from vitamins A and D, in the diet might have been responsible for the hepatic, muscular and nervous changes. This is not felt to be likely, however, as earlier experiments had shown that the addition of linoleic acid to the diet did not prevent the hepatic fibrosis and this was accompanied in one rabbit by marked muscle wasting.

No nervous complications of any sort have been described as occurring in vitamin E deficiency in the rabbit [Pappenheimer, 1948] although this animal has been studied by several groups of authors in the past 20 years. On the other hand Ringsted [1935] and Einarson and Ringsted [1938] have described in rats neuromuscular changes somewhat similar to those in our rabbit 118. Their animals were fed on a diet that contained fat but was free from vitamin E. Their results, however, could only be produced with difficulty and the development of the changes was a much more chronic process than usually encountered in the rabbit myopathy. The similar curious selection of the posterior roots and columns together with the muscles, and only later involvement of the motor nerves, is a matter of interest. One difference, however, from our findings, is the presence in the nerve cells of Einarson and Ringsted's rats of acid-fast granules which could not be detected in our animals. This difference might be the effect of species and perhaps of chronicity. It is thus conceivable that we have produced the same condition in the rabbit but have been frustrated in regularly encountering the full picture by the early death of our animals. The fact that two of the rabbits receiving α-tocopherol developed the same minimal nervous lesion tends to discount the essential importance of this vitamin in the process.

The process with regard to the myopathic lesions is less clear. The changes are typical in almost every way, of the myopathy produced in animals on vitamin E deficient diets by other workers with the important exception that our lesions are rather less massive and extensive and rather more chronic. Pappenheimer [1943] gives as the minimal vitamin E requirements of the rabbit as 0·3 mg./kg./day, while Mackenzie *et al.* [1940] have put it as high as 0·6–1·6 mg./kg./day. Our dose of approximately 1 mg./kg./day was therefore about the minimal level, but nevertheless gave no apparent protection from muscular damage.

Changes in skeletal and cardiac muscle on a vitamin E deficient diet are well known in young rabbits [Pappenheimer, 1940; Mackenzie and McCollum, 1940; Bragdon and Levine, 1949] but no record has been found of any such changes in adult rabbits. Furthermore Pappenheimer studied the brain and spinal cord in his deficient animals and found no alterations of significance. Also the reaction of Pappenheimer's young rabbits differed from those seen in the animals described here in that growth appeared to proceed normally for 2–6 weeks after the commencement of the diet, as if stores of α-tocopherol were being utilized. In contrast our animals began to lose weight within a few days of taking the diet. In our experiments the vitamin was given intramuscularly so that problems of intestinal absorption do not arise. Thus the association with nervous lesions, the differing natural history and the lack of response to parenterally administered vitamin E must together suggest that this experimental syndrome is not the same as that associated with specific vitamin E deficiency. The lack of specificity of this type of muscle change is well known, and must not be allowed undue importance.

It is concluded therefore that although a syndrome superficially resembling vitamin E deficiency myopathy can be produced in rabbits on a "fat free" casein and sucrose diet, there are good reasons for believing that the mechanism of production is different and vitamin E deficiency plays little or no part in it.

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THE OSMOTIC PRESSURE (FREEZING POINT) OF HUMAN SWEAT IN RELATION TO ITS CHEMICAL COMPOSITION.*

By R. ADAMS,† R. E. JOHNSON ‡ and F. SARGENT, II.§ From the Department of Physiology, University of Illinois, Urbana, Illinois, U.S.A.

(*Received for publication 17th December 1957*)

We have studied the osmolarity (freezing point) and chemical composition of specimens of forearm sweat collected from ninety-five young men who walked outdoors in moist heat on five occasions wearing an elbow-length glove on each arm. Chemical estimations included sodium, potassium, ammonia plus urea, chloride, lactic acid, acetone plus acetoacetic acid plus beta hydroxybutyric acid, creatinine, and acidity.

Compared with serum, sweat was hypotonic in total osmolarity, sodium and chloride; about isotonic in urea, ketone bodies, and creatinine; and hypertonic in potassium, ammonia, lactate and hydrogen ion. Ketone bodies did not increase with increasing ketonemia. In a majority of specimens, measured osmolarity was greater than the sum of the osmotic effects of measured constituents; in about 15 per cent of all specimens the discrepancy was 25 per cent or more of total osmolarity. An "unmeasured osmol" in sweat is postulated, possibly an organic acid of small dissociation constant.

Because of large variability within and between subjects, correlation between measured osmolarity and individual constituents was not close.

There is general agreement that with increasing rate of sweating, concentrations of sodium and chloride increase, while at the same time those of potassium, ammonia and urea decrease. We would add a decrease in total osmolarity and "unmeasured osmol". Statistical concordance with all these changes is afforded by a model which postulates that total sweat is made up of two kinds, each of different, but constant, composition. The first would predominate at low rates of sweating, but increase relatively slowly with increased total rate. It would be relatively low in concentration of sodium and chloride, high in total osmolarity, "unmeasured osmol", potassium, ammonia, and urea. The second would increase rapidly in relative volume with increased total rate until it predominated at high rates of sweating. In composition it would be opposite to the first type. Total concentrations at any given total rate of sweating would be the resultant of the contributions of these two kinds of sweat.

INTRODUCTION

WATER metabolism and osmotic regulation are so closely related that they can hardly be studied separately; so it is remarkable that the various physical properties of sweat have received little attention. In fact, only twelve investigators give direct measurements of the freezing point of human sweat,

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* This investigation was under contract AF 18(600)—80 between the U.S. Air Force and the University of Illinois.

† The substance of this paper was submitted in partial fulfilment of the requirements for the degree M.S. in Physiology, University of Illinois, 1957.

‡ U.S. National Science Foundation Senior Postdoctoral Research Fellow, Department of Physiology, Edinburgh University.

§ John Simon Guggenheim Memorial Foundation Fellow, M.R.C. Unit for Research on Climate and Working Efficiency, Department of Human Anatomy, Oxford University.

and these are summarized in Table I. The osmotic pressure of sweat is not discussed at all in the reviews of Kuno [1934, 1956] and Robinson and Robinson [1954], and mentioned only in passing in the books of Rothman [1954] and Fiedler [1955].

Presumably, previous observers would agree on three general conclusions. First, human sweat is usually hypotonic, sometimes markedly so, if −0·55° C. be taken as a normal value for serum's freezing point. Secondly, sweat's osmotic pressure varies from subject to subject, from one place on the body to another, and from time to time as does its chemical composition. Finally, osmolarities are lowest in sweat collected in impermeable gloves or sheets, and highest in sweat scraped from the skin. There would be no unanimous agreement on the correlation between osmotic pressure and chemical composition. Ardin-Deltiel [1900] and Bogdan [1907] asserted that sodium chloride, the major contributory osmol, could be used to predict freezing point depression. Van Heyningen [1949] measured chloride, lactic acid and urea and Amatruda and Welt [1953] sodium, potassium, ammonia and urea in sweat. Each drew up sophisticated empirical equations relating osmotic pressure to these independent variables. Strauss [1904] and Adams [1957] were not satisfied that close correlation existed between freezing point and the constituents that they measured.

We have measured the freezing point of 428 specimens of sweat collected in elbow-length gloves from ninety-five men; in these specimens we also estimated the concentration of sodium, potassium, urea plus ammonia, chloride, lactate, ketone bodies and creatinine. A statistical study of these two sets of data has enabled us to generalize on the relationships between rate of sweating, osmotic pressure, and chemical composition.

METHODS

Subjects.—For six consecutive weeks, ninety-five young men, average age 18 years, range 17–20, were kept under constant observation. All were clinically free from disease. Twenty-five were Negroes, the rest white. Daily activity and daily intake of food and water were measured and all urine and fæces were collected for chemical analysis. During the first and last two weeks, an adequate diet with unlimited fluids was provided, and daily work was moderate. During the middle two "experimental" weeks, each of twenty different regimens was imposed rigidly on separate small sub-groups, covering the possible combinations of calories (3000, 2000, 1000 and 0); water (unlimited, limited to 910 ml./day); protein/carbohydrate/fat ratio (low to high with respect to each); osmotic intake (low to high in protein or salt); and daily work (3 miles or 12 miles marching per day). Twelve of the ninety-five served as controls, living throughout the 6 weeks on an abundant diet of fresh and frozen foods, with unlimited water. All subjects received daily a multi-vitamin preparation.

Production of Sweat.—On one afternoon each week after lunch during the first 2 and last 2 weeks, and once during the "experimental" period, groups of about twenty-five subjects reported to a barracks-laboratory, where they stripped, weighed, and reclined for 25 min. while pulse rates, rectal temperature and skin temperature were measured. At a fixed pace of 6·25 km./hr., they marched for 1 hr. outdoors around a circular level track of 0·7 km. circumference, partly in sun, partly in shade. After the first week, the weather was hot and moist; for the five marches, basic

TABLE I.—DIRECT MEASUREMENTS OF FREEZING POINT (OSMOLARITY) OF HUMAN SWEAT

Reference	Number of specimens	Freezing point		Method of stimulation Method of collection Kind of subject
		Mean °C.	Extremes °C.	
Ardin-Deltiel [1900]	15	−0·24	−0·08 to −0·46	Hot air bath. Collection not described. Normal men.
Mairet and Ardin-Deltiel [1900 *a*, *b*, *c* and *d*]	11	−0·24	−0·08 to −0·52	Same methods as above. Normal men; patients with epilepsy and general paralysis.
Strauss [1901]	21	−0·385	−0·13 to −0·64	Hot air bath. Rubber glove. Patients with nephritis, rheumatism, or anemia.
Strauss [1904]	80	−0·42	> −0·30 to −0·71	Twenty-five patients with nephritis, fifty-five with neuritis or rheumatism.
Brieger and Diesselhorst [1903 *a*]	50	−0·61	−0·32 to −1·00	Radiant heat. Sweat scraped into beaker. Fifteen normal men; nineteen patients with rheumatism; two with nephritis; nine with "neurasthenia".
Brieger and Diesselhorst [1903 *b*]	3 3*a* 3*b*	−0·281 −0·394 −0·450	−0·270 to −0·297 −0·332 to −0·422 −0·412 to −0·472	Compared glove sweat with (*a*) sweat dripped into beaker and (*b*) scraped off. One normal man.
Brieger and Diesselhorst [1903 *b*]	7 7*a*	−0·327 −0·381	−0·267 to −0·427 −0·268 to −0·497	Compared glove sweat with (*a*) impermeable sheet. Patients with neuritis, bronchitis, lues or nephritis.
Bendix [1904]	6	−0·31	−0·16 to −0·78	Hot air bath. Whole body covered with impervious sheet. Patients with nephritis.
Bogdan [1904]	22	−0·31	−0·24 to −0·37	Hot air bath; or inhalation of moist warm air; or drinking hot tea. Sweat scraped from chest near sternum. Seventeen normal men and five women.
Tarugi and Tomasinelli [1907–8]	9	−0·52	−0·42 to −0·58	Radiant heat. Sweat dripped into cylinder from trunk. Normal men.
Marchionini [1928]	20	−0·45	−0·27 to −0·66	Radiant heat. Sweat scraped into beaker from face, hands and feet. Subjects not described.
Couraud [1935]; and Delaunay and Couraud [1935]	Not given	−0·3	−0·2 to −0·5	Hot air bath. Sweat collected in rubber bag. Subjects not described.
Lifson and Lorber [1945]	21	−0·341*	−0·173* to −0·590*	Stimulation, collection, subjects not described. Used Hill-Baldes osmometer.
van Heyningen [1949]; and Weiner and van Heyningen [1949]	150	−0·42*	−·023* to −0·61*	Physical work in moist heat. Sweat collected in arm bags from sixteen healthy men.
Lichton [1957 *a* and *b*]	25	−0·13*	−0·09* to −0·20*	Physical work in moist heat. Forearm rubber gauntlet. One acclimatized subject.
Adams [1957]; and Johnson, Adams and Sargent [1956]	428	−0·302*	−0·094* to −0·688*	Walking in moist heat. Elbow length rubber gloves. Ninety-five normal men.

* Authors' original tabulations in mols. Recalculated to freezing point with factor −1·84° C. for 1000 mOsm/l. at dilutions usually encountered in sweat.

effective temperatures averaged 20° C., 27° C., 26° C., 25° C. and 25° C., respectively. After the walk, the men returned to the laboratory barracks, where measurements were repeated. No food, water or smoking were permitted immediately before or after, or during the walk.

Collection of Sweat.—On the previous night, both arms were shaved up to the elbow. Before the march, the arms were dipped in distilled water, and dried with paper towels. An obstetrical rubber glove, reaching to the elbow, was worn on each arm during the walk, held just below the elbow with a broad rubber band. For each group, one subject wore at his belt two such gloves, each containing 100 ml. of distilled water. These "glove blanks" were analyzed subsequently along with the sweat; the gloves contributed nothing of significance to the contents. After the march, all gloves were emptied into measuring cylinders, a portion of sweat was kept for immediate qualitative analysis, and the rest frozen without added preservative for subsequent quantitative analysis.

Chemical Analyses.—All methods were validated by recovery studies with both water and sweat. Along with each batch of ten to twenty samples, there was an appropriate blank and standard. The various methods were: (1) sodium and potassium by flame photometry [Baird Associates, 1953] with an internal standard of lithium nitrate and without prior ashing of specimens; (2) chloride by thiocyanate titration [Keys, 1937]; (3) lactic acid [Barker and Summerson, 1941]; (4) acetone, acetoacetic acid and beta-hydroxybutyric acid [Michaels, Morgan, Liebert and Kinsell, 1951] modified so as to give 100 per cent oxidation of beta-hydroxybutyric acid under the catalytic influence of metaphosphoric acid; (5) creatinine as "true chromogen" [Haugen and Blegen, 1953]; and (6) urea plus ammonia by nesslerization following treatment with urease [Consolazio, Johnson and Marek, 1951]. Because urea contributed 80 per cent and ammonia 20 per cent of the total (urea plus ammonia) nitrogen in nine specimens, S.D. ±5 per cent, urea and ammonia were calculated in all specimens separately by application of those percentages. From twelve successive analyses of the same specimen of sweat, replicate reliability was: sodium, 0·05 m.equiv/l.; potassium 0·07 m.equiv/l.; chloride, 0·2 m.equiv/l.; lactic acid 0·1 mM/l.; total ketone bodies 0·04 m.equiv/l.; creatinine, 0·01 mM/l.; and urea plus ammonia, 0·15 mM/l.

Osmometry.—To estimate freezing point, we used a commercial thermistor device (Fiske Associates, Inc., Boston, Massachusetts) which is sensitive to at least 1×10^{-4}° C.; this is ten times as sensitive as the conventional Beckman thermometer. For reproducibility to 0·5 mOsm/l., a rigid routine is essential. Of especial importance are accurate measurement of 2·0 ml. of sample; identical rates of cooling before freezing; and constant final temperature at which the super-cooled specimen is caused to crystallize. Aqueous solutions of NaCl in the range 0–0·5 molal provided calibration points, and conversion from molality to osmolality was made from data on molal freezing point depression as given in the International Critical Tables [1928].

Definitions.—Confusion could be prevented in the literature of osmotic regulation, if the dial of the thermistor device, which we and many others use, were marked in arbitrary units, or degrees C., instead of osmols, with the implication of rigorous physico-chemical usage. There are several reasons why the convention of expressing osmotic concentration as milliosmols per kg. water is unnecessarily pedantic. First, all that the instrument measures is freezing point. Secondly, the assumption underlying conversion of freezing point to osmolality by a conversion factor of −1·86° C./mol may be acceptable for a single non-ionized molecule at infinite dilution, but it may not be true for a complex mixture, such as sweat, of strong electrolytes, weak electrolytes and non-electrolytes. Thirdly, all chemical concentrations commonly used in physiology are on a molar, not a molal basis, and uniformity is best preserved by continuing to use molarities for all measurements, including osmotic concentration. Finally, the distinction becomes meaningless for physiological purposes when calibration curves are made with solutions of sodium chloride. The most concentrated specimens of urine are about 0·7 molal (sodium chloride equivalent); serum is about 0·15 molal; sweat is usually about 0·075 molal. When salt is mixed with water in these concentrations, the difference between molal and molar is insignificant. For example, at 18° C. a solution 0·700 molal in sodium chloride is 0·697 molar; a solution 0·150 molal in sodium chloride, 0·150 molar; and a solution 0·075 molal in sodium chloride, 0·075 molar. In short, within the physiological range and with reference solutions made from sodium chloride, molality is interchangeable with molarity. We urge that osmometrists should record their actual measurement, which is freezing point; specify what formula is used to convert to osmotic pressure; and recognize that their "osmolality" or "osmolarity" is a derived, not a primary, datum. Further, we urge the general adoption of molarity for the sake of simplicity.

Calculations.—The basic arithmetical assumption of some of our calculations has been that freezing point is directly correlated with the arithmetic sum [unionized molecules] + [ionizable molecule] × (1 + valence of cation) × (activity coefficient). This assumption was tested with a specimen of "synthetic sweat", made by dissolving in water NaCl, KOH, $(NH_4)OH$, racemic lactic acid, and urea, so that the final molal concentrations were: Na and Cl, 0·180; K, 0·030; ammonia plus ammonium, 0·040; lactic acid plus lactate, 0·070; and urea, 0·070. The final pH was 4·3; the final total molality, 0·570; and the final total osmolality, corrected for dissociation and activity coefficients, 0·513. Dilutions were made to give final osmolalities, representing very dilute to very concentrated samples of sweat, of 0·057, 0·114, 0·228 and 0·342. Freezing point was linearly related to molality; and calculated osmolality at all dilutions was the same as sum of constituents, corrected for dissociation and activity coefficients, within ±2 mOsm/l. As a further check on the assumption that freezing point is linearly related to sweat concentration, three different samples of concentrated real sweat were diluted serially. Within ± 2 mOsm/l., the freezing point was inversely proportional to dilution in the dilutions 1:1, 1:2 and 1:4. We concluded that within the usual range of sweat concentrations, freezing point is correlated with effective osmotic concentration.

On occasion, it became necessary to distinguish between total lactic acid and lactate, and between total ammonia and ammonium ion. For these calculations, the pK of lactic acid was taken to be 3·857 and the pK of ammonium hydroxide 4·728. The pH was known for each specimen, the grand mean being 4·85.

Statistical Approach.—Only conventional statistical techniques were used in handling the data. Distribution curves were not smoothed. Curve fitting of linear plots was by the method of least squares. In deciding whether there is a consistent correlation between the total osmolarity and any combination of the chemical constituents, we had recourse to the digital computer "Illiac".* The program (K–2–135) permitted multi-correlation, with analysis of variance, analysis of covariance, mean and standard deviation. Statistically, the control subjects and experimental subjects proved to be different populations, and their data were kept in separate groups for almost all kinds of numerical study. In deciding whether or not total osmotic concentration was related to sum of constituents, each sample was treated separately. Sum of constituents, anionic sum, cationic sum and percentage of total osmolarity contributed by sum of constituents, were computed for each specimen, and then averages and measures of variance were calculated for the populations of these derived data.

The contribution we make to sweat gland physiology is a statistical study of the freezing point of sweat collected from many subjects studied a few times. Our results may contribute valuable information on the limits of variability of the composition of arm sweat collected in gloves during physical work in moist heat; and on phenomena common to most individuals in a large population. Our studies were not planned to contribute to knowledge on individual sweat glands.

* Professor R. A. Meagher, Head of the Computer Laboratory, University of Illinois, kindly provided statistical routines and time on the "Illiac".

RESULTS

Osmometric and Chemical Data.—Overall mean values for the experimental groups (eighty-three subjects, five walks, 374 specimens analyzed completely) and the control groups (twelve subjects, five walks, fifty-four specimens analyzed completely) are given in Table II, to which are added values for a "typical serum". Sweat is not much more constant in composition than urine; measures of variance would be somewhat inappropriate and are therefore omitted. Distribution curves, however, do give a realistic approach to the variability of sweat (Table III). In comparison with serum, our specimens of sweat tended uniformly to be hypertonic in ammonia, potassium,

TABLE II.—MEAN VALUES, SWEAT OSMOLARITY AND CHEMISTRY, COMPARED WITH VALUES FOR "TYPICAL" SERUM

Measurements and units	Sweat of experimental subjects	Sweat of control subjects	"Typical" serum*
Osmolarity, mOsm/l.	163	108	286
Sodium, m.equiv/l.	41	27	135
Potassium, m.equiv/l.	12	8	4·7
Ammonia, mM/l.	10	7	(0·1)†
Chloride, m.equiv/l.	38	26	103
Lactic acid, m.equiv/l.	20	17	(1 – 3)†
Total ketone bodies, mM/l.	0·6	0·4	0·6
Urea, mM/l.	19	15	14
Creatinine, mM/l.	0·1	0·1	0·1
pH	4·8	4·9	7·3

* Modes for all subjects at rest, pre-period.

† Not measured in these subjects, but others. Blood lactic acid levels are not raised at the speed of walking used.

TABLE III.—STATISTICAL INFORMATION ON OSMOLARITY, CATIONS, ANIONS AND UREA IN SWEAT

Measurement and units	Experimental subjects *				Control subjects †			
	Mean	Mode	S.D.	Type of distribution	Mean	Mode	S.D.	Type of distribution
(*a*) Osmolarity								
Total osmolarity (mOsm/l.)	163	145	50	Gaussian	108	96	24	Gaussian
Sum of Constituents (percentage total osmol)	88	91	12	Gaussian	93	96	9	Gaussian
(*b*) Cations								
Sodium (m.equiv/l.)	41	36	16	Poisson	27	23	12	Poisson
Potassium (m.equiv/l.)	11·7	9·8	4·0	Gaussian	8·5	8·4	2·2	Gaussian
Ammonia (m.equiv/l.)	9·6	8·8	2·9	Gaussian	7·4	7·5	1·7	Gaussian
(*c*) Anions and Urea								
Chloride (m.equiv/l.)	38	30	15	Poisson	26	24	10	Gaussian
Lactic acid (m.equiv/l.)	20	21	8	Irregular	17	12, 18	7	Bimodal
Urea (mM/l.)	19	17	6	Gaussian	15	15	3	Gaussian

* Three hundred and seventy-four specimens were collected in five different tests and completely analyzed.

† Fifty-four specimens were collected in five different tests and completely analyzed.

and lactic acid; about isotonic in urea; and hypotonic in sodium, chloride, and osmolarity. In all these measurements, the control subjects, who were always well fed and well watered, tended to be lower than the experimental subjects, whose dietary restrictions were close. In both populations, variability was marked. The ketone bodies comprise a quantitatively very small, but nonetheless interesting, portion of the sweat. There is no published information on these substances in sweat, but we predicted that there would be a considerable concentration, and that in ketosis it would increase. Both predictions were erroneous (Table IV). The ketogenic regimens were starvation and two different mixtures, each high in fat. The non-ketogenic regimens were pure carbohydrate and another mixture moderately high in carbohydrate and protein, low in fat. The control ration provided an abundance of meat, bakery products, dairy products, fruits and vegetables. At low serum concentrations total ketone bodies were rarely higher in sweat than in serum; with increasing serum concentrations there was no corresponding increase in the sweat concentration. Quantitative estimation of the individual ketone bodies in nineteen fresh specimens gave average figures for acetone, 8 per cent of total; for acetoacetic acid, 42 per cent; and for beta-hydroxybutyric acid, 50 per cent. The contrast between lactate and ketone bodies in sweat is great. Serum concentrations are about the same, 1 mM/l., but sweat lactate averages about 20 mM/l., and sweat ketone bodies less than 1 mM/l. Lactate must either be produced by the sweat gland, or actively concentrated. Ketone bodies must either be consumed by the sweat gland, or actively prevented from diffusing. Creatinine, even less concentrated in sweat than ketone bodies, cannot contribute appreciably to the osmolarity, and is not included in the distribution curves.

Table IV.—Total Ketone Bodies in Sweat from Ketotic and non-Ketotic Subjects, compared with Serum Concentrations

Regimens and subjects	Serum concentrations of total ketone bodies, mM/l.						
	0–0·59	0·6–0·99	1–1·99	2–2·99	3–3·99	4–4·99	5 and over
A. Three ketogenic regimens							
No. of subjects . . .	5	10	10	9	4	1	1
Mean sweat concentration .	1·02	0·66	0·71	0·81	0·49	0·74	1·08
B. Three non-ketogenic regimens							
No. of subjects . . .	26	15	3	..	..	..	..
Mean sweat concentration .	0·68	0·99	0·54	..	..	..	..
C. One control regimen							
No. of subjects . . .	7	5	..	..	..	..	..
Mean sweat concentration .	0·28	0·58	..	..	..	..	..

Lack of Correlation between Osmolarity and Concentration of Individual Constituents in Sweat.—Although the mean contribution to total osmolarity was about 25 per cent for both sodium and chloride, 14 per cent for both lactic acid and urea, and 7 per cent for both potassium and ammonia (Table V), these percentages were very far from constant, nor were they predictable in any given specimen.

Correlation coefficients were calculated among all the constituents here considered for both experimental subjects and control subjects (Table VI). Correlations between urea and ammonia may be discounted; they were auto-correlated because of the constant factor used in calculating them from the combined value for urea plus ammonia nitrogen. A good correlation existed between sodium and chloride. Only fair to poor correlations existed between osmolarity and the individual chemical constituents. All other correlations among chemical constituents were poor. A tendency is apparent for sodium and chloride to vary inversely with potassium, urea and ammonia.

TABLE V.—CONTRIBUTION MADE TO SWEAT OSMOLARITY BY VARIOUS CHEMICAL CONSTITUENTS

Constituent	Per cent osmolarity			
	Experimental subjects *		Control subjects †	
	Mean	Range	Mean	Range
Sodium .	26	10–46	25	12–40
Chloride .	23	19–39	24	12–40
Lactic acid .	13	2–32	16	2–30
Urea . .	12	6–27	14	7–23
Potassium .	7	3–14	8	3–13
Ammonia .	6	3–13	7	3–11
Unaccounted	13	. .	6	. .

* Three hundred and seventy-four specimens were collected in five different tests and completely analyzed.

† Fifty-four specimens were collected in five different tests and completely analyzed.

TABLE VI.—CORRELATIONS * AMONG OSMOTIC CONCENTRATION AND THE CONCENTRATIONS OF MAJOR CHEMICAL COMPONENTS OF SWEAT

Measurement	Osmolarity mOsm/l.	Sodium m.equiv/l.	Potassium m.equiv/l.	Chloride m.equiv/l.	Urea mM/l.	Ammonia m.equiv/l.	Lactic acid m.equiv/l.
Osmolarity .		0·630	0·640	0·606	0·550	0·554	0·204
Sodium .	0·744		0·009	0·931	−0·021	−0·016	−0·011
Potassium .	0·340	−0·187		−0·040	0·654	0·656	0·366
Chloride .	0·650	0·936	−0·326		−0·013	−0·008	−0·038
Urea .	0·289	−0·171	0·640	−0·242		0·999	0·118
Ammonia .	0·289	−0·171	0·670	−0·242	1·000		0·120
Lactic Acid .	0·294	0·033	0·414	−0·088	−0·288	0·288	

Figures above the diagonal refer to 374 specimens from experimental subjects and figures below to fifty-four specimens from control subjects.

* Correlation coefficients calculated by the digital computer "Illiac" according to programme K–2–135, multi-correlation with analysis of variance.

Because of these poor correlations, one would not expect osmolarity to be calculable with any great accuracy by means of a simple equation in which constant factors are used for transforming chemical composition to osmolarity. One such equation was used by Amatruda and Welt [1953], without proof of its validity. They did not measure directly the osmotic pressure, but used the equation:

$$\text{osmolarity} = 2(\text{Na}) + 2(\text{K}) + 2(\text{NH}_3) + (\text{urea}).$$

Their equation assumes that lactic acid is quantitatively accounted for by ammonia and potassium as lactate, and chloride as sodium chloride. For ninety-nine of our specimens, chosen at random, direct measurement gave a mean of 161·6 mOsm/l. and the equation of Amatruda and Welt [1953] a mean of 136·6. Analysis of variance gave an F value of 106·7, or a probability of less than 0·01 that these were chance mean differences. We cannot recommend their equation for general use. Another such equation was used by van Heyningen [1949]. She measured freezing point and calculated an empirical equation of the form:

$$\text{osmolarity} = 2(\text{Cl}) + 2{\cdot}18\ (\text{lactate}) + 2{\cdot}11\ (\text{urea}).$$

This equation assumes an equivalence between sodium and chloride, potassium plus ammonia and lactate, and an empirical effect of urea. We were unable to repeat her original calculations because she did not report the actual freezing points. Her chloride concentrations average 108 m.equiv/l., much higher than any other recent observers have reported [Robinson and Robinson, 1954]. Analysis of variance on fifty-one randomly chosen specimens of ours with a mean actually measured osmolarity of 146·8 and a mean by van Heyningen's equation of 120·6, showed no significant correlation ($F = 22{\cdot}32$, P less than 0·01). We cannot confirm the general validity of the assumptions of her equation, even though they may have fitted her own specimens. Lichton [1957 *a* and *b*] reported a correlation coefficient of 0·834 between measured osmolarity and sweat sodium; that a correlation exists we agree, although ours is not as high. He did not construct an equation to predict osmolarity from chemical constituents. We are inclined to agree with his conservatism, because of data now to be presented.

Evidence of an "Unmeasured Osmol" in Sweat.—The sum of sodium, potassium, ammonia, chloride, lactic acid and urea did not in a majority of our specimens equal the observed osmolarity (Table III). In about 15 per cent of all samples as much as 25 per cent of total osmolarity remained to be accounted for. Average deficits were 6 per cent for control subjects and 13 per cent for experimental subjects. These calculations involve the conservative, but unlikely, assumption that all the electrolytes are totally ionized. If activity coefficients were assumed to be 0·93 for sodium and potassium chloride and a dissociation of 85 per cent for ammonium lactate at pH5 and at usual concentrations in sweat, then the discrepancy between observed osmolarity and the sum of the constituents under consideration would be even larger than it is in Table III.

Further evidence that there is an "unmeasured osmol" in sweat, comes from a consideration of osmolarity and rate of sweating. Osmolarity was generally lower with higher rates of sweating (Table VII), and in fact the plot of osmolarity on rate of sweating can be fitted approximately with an exponential function. A plot of measured osmolarity on percentage of osmolarity

TABLE VII.—OSMOLARITY OF SWEAT AND RATE OF SWEATING IN GLOVE

Intervals of sweat rates in glove ml./hr.	Specimens number	Mean osmolarity mOsm/l.
	Twelve control subjects, all five periods	
0–19	8	136
20–39	19	111
40–59	12	101
60–79	9	90
80–99	2	83
	Eighty-three experimental subjects,	
0–19	37	178
20–39	53	157
40–59	44	146
60–79	13	141
80–99	4	124

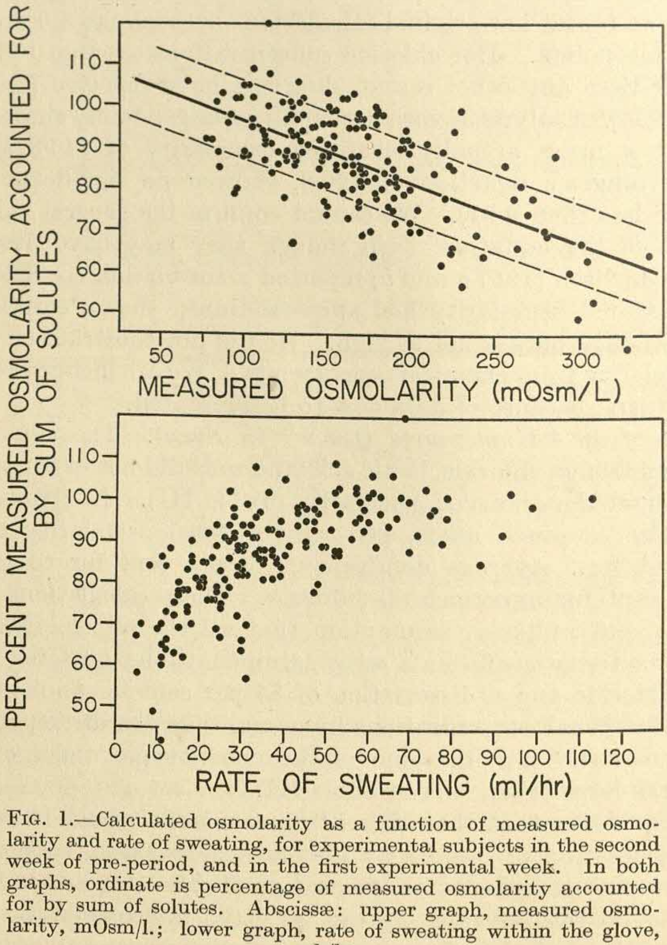

FIG. 1.—Calculated osmolarity as a function of measured osmolarity and rate of sweating, for experimental subjects in the second week of pre-period, and in the first experimental week. In both graphs, ordinate is percentage of measured osmolarity accounted for by sum of solutes. Abscissæ: upper graph, measured osmolarity, mOsm/l.; lower graph, rate of sweating within the glove, ml./hr.

accounted for by the sum of the constituents (fig. 1, upper) suggests that with increasing osmolarity, there is an increased concentration of something, other than sodium, potassium, ammonia, chloride, lactate or urea, which contributes significantly to the osmolarity. By the method of least squares, the regression line was (per cent of measured osmolarity accounted for by sum of solutes) $= 111 - 0{\cdot}15$ (measured osmolarity). For this line the standard deviation of Y on X was 9·3, and the correlation coefficient was $-0{\cdot}65$ ($P < 0{\cdot}001$).

TABLE VIII.—COMPARISON BETWEEN TOTAL "OSMOTIC DEFICIT" AND "IONIC DEFICIT" IN SWEAT

Observation	Experimental subjects	Control subjects
A. Number of specimens (all weeks)	374	54
B. Measured osmolarity, mOsm/l.		
Mean	163	108
Maximum	374	194
C. Sum of constituents, mM/l.		
Mean	145	103
Maximum	275	129
D. "Osmotic deficit", B–C		
Mean	18	5
Maximum	99	65
E. Sum of cations, m.equiv/l.		
Mean	62	44
Maximum	142	93
F. Sum of anions, m.equiv/l.		
Mean	55	43
Maximum	132	94
G. "Anionic deficit", m.equiv/l.		
Mean	7	1
Maximum	40	17
H. pH (indicator paper)		
Mean	4·8	4·9

With low rates of sweating, the contribution of this "unmeasured osmol" becomes increasingly important, so that at very low rates it may account for 40–50 per cent of the total osmolarity (fig. 1, lower); at high rates of sweating, its effect is small or absent.

We have not yet characterized the "unmeasured osmol" chemically. Indirect quantitative and qualitative evidence leads in the direction of an organic acid of quite high pK.

The quantitative evidence rests on a comparison of the total "osmotic deficit" against the total "ionic deficit" (Table VIII). For each specimen, "osmotic deficit" was calculated as the difference between measured osmolarity and the sum of sodium, potassium, ammonium, chloride, lactate and urea. "Ionic deficit" was calculated as the difference between sum of measured cations (*i.e.* sodium, potassium and ammonium) and sum of measured anions (*i.e.* chloride and lactate). There was some difference in those values between experimental subjects and control subjects. To accentuate the discrepancy, maxima are given as well as means. For both experimental subjects and controls, the "osmotic deficit" was larger than the "anionic deficit". The mean sweat pH was about the same for both groups. An organic acid of high pK could produce these results because at pH5 it would be ionized only partially. Therefore, its total osmotic effect would be greater than its ionic effect.

Three qualitative observations may or may not relate to the "unmeasured osmol". Firstly, almost all specimens of sweat, when tested by the nitroprusside reaction of Rothera, gave a blue or light blue-green colour. This can be obtained with an alpha-ketonic acid, in contrast to the purple yielded by beta-ketonic acids or acetone. Secondly, when tested for albumen with sulfosalicylic acid, no specimen of sweat yielded a precipitate, but many of them turned pink. We do not know what compounds react this way. Finally, a pigment with maximal absorption at 400 millimicrons was detectable in our specimens, in greatest concentration when the percentage of "unmeasured osmol" was greatest. Perhaps we were dealing with the urocanic acid which was identified in sweat by Ženišek and Kral [1953], and suspected by Vandenbelt *et al.* [1954]. This is doubtful, for urocanic acid occurs in such low concentrations.

DISCUSSION

The "unmeasured osmol" accounts in extreme cases to almost half the total osmolarity of sweat, and as much as 90 mOsm/l. Therefore, we are looking for something present sometimes in large concentrations, and unlikely to be any of a very considerable number of substances measured by us or others [see Fiedler, 1955; Kuno, 1956; Mitchell and Edman, 1951; Robinson and Robinson, 1954; and Rothman, 1954]. Carbon dioxide is not probable. When three specimens were exposed separately to pure nitrogen and to 80 per cent CO_2 plus 20 per cent N_2, the average increase caused by this quite unphysiological partial pressure was only 4·0 mOsm/l. No more than 2 mOsm/l. could be contributed by calcium, which we did not measure, and less than 2 mOsm/l. by the sum of inorganic phosphorus, sulfur, iodine, bromine, fluorine, magnesium, copper, manganese and iron. The sum of amino acids, including citrulline and urocanic acids, and uric acid, creatine and creatinine is probably less than 2 mM/l. Of lipids and their derivatives, only very small amounts occur, including also cholesterol, short chain fatty acids, ethyl alchohol, glycerine and ketone bodies. Vitamins and hormones occur only in micromolar concentrations. Pyruvic acid, citric acid and glucose have not been reported over 0·5 mM/l. Except for lactic, pyruvic and citric acids, no study has been reported on compounds of the glycolytic and citric acid cycles; and except for ammonia, urea, and citrulline on intermediates of the arginine cycle. Among the compounds of these various cycles may be found our "unmeasured osmol".

Most sweat physiologists would presumably agree on a few general points:

(*a*) Even under identical conditions of work, environment and dietary regimen, there are individual differences in the composition of sweat from time to time, and from subject to subject, and from one area of the body to another.

(*b*) The concentrations of sodium and chloride are equal.

(*c*) The concentrations of ammonia, potassium and lactic acid in sweat are hypertonic to serum.

(*d*) The concentration of urea in sweat is generally almost isotonic to serum.

(*e*) The concentrations of sodium and chloride in sweat are hypotonic to serum.

(*f*) When sweat rate increases, the concentrations of sodium and chloride usually rise, and those of potassium, ammonia and urea usually fall.

To these we would now add:

(*g*) The osmotic concentration of sweat is hypotonic to serum.

(*h*) There is in many specimens of sweat a substance (or substances) which contributes to the osmolarity, sometimes substantially, and which is not sodium, potassium, ammonia, chloride, lactate, urea or ketone bodies.

(*i*) When sweat rate increases, osmotic concentration usually diminishes, as does the percentage of "unmeasured osmol" in the sweat.

Most workers have assumed that for a given area of the skin, the composition of the sweat produced by each individual gland varies in the concentration of its chemical constituents, especially with fluctuation in the rate of sweating, and have theorized on the secretory and reabsorptive functions of various parts of the gland [*e.g.* Lobitz and Mason, 1948; and Schwartz, Thaysen and Dole, 1953]. Mathematically speaking, however, there is no necessity to postulate any variability in the quantitative composition of sweat from individual very small areas to satisfy the observations (*c*) through (*i*) listed above. A statistical model can be made in which the only postulates are that in any given area, there are two kinds of sweat, one increasing in volume slowly, the other increasing rapidly, that "sweat A" is somewhat low in sodium and chloride, but high in potassium and ammonia, urea, lactic acid, "osmol X" and total osmolarity; and that "sweat B" is somewhat high in sodium and chloride, but low in potassium, ammonia, lactic acid, urea, "osmol X" and total osmolarity. A numerical example of the consequences of this model is given in fig. 2. With increasing rates of sweating, "sweat B" will dilute the mixture, and the result will be, with increased sweating, a rise in sodium, chloride, and percentage of total osmolarity accounted for by the sum of Na, Cl, K, NH_3, lactate and urea; and a fall in potassium, urea, ammonia and total osmolarity. All these consequences are in accord with established behaviour of sodium, chloride, potassium, ammonia and urea. They are also in accord with our own observations on osmolarity and the "unmeasured osmol". Adumbrations of the "two sweat hypothesis" are to be found in publications by Kuno's school [Kuno, 1956] not only on the distinction between apocrine and eccrine sweat glands, but also on a distinction between eccrine sweat glands into those responding to emotional and those responding to thermal stimuli. His "skin chloride shift in sweating" is a mathematical model which is in accord with observed behaviour of chloride with sweating, but it does not cover other solutes.

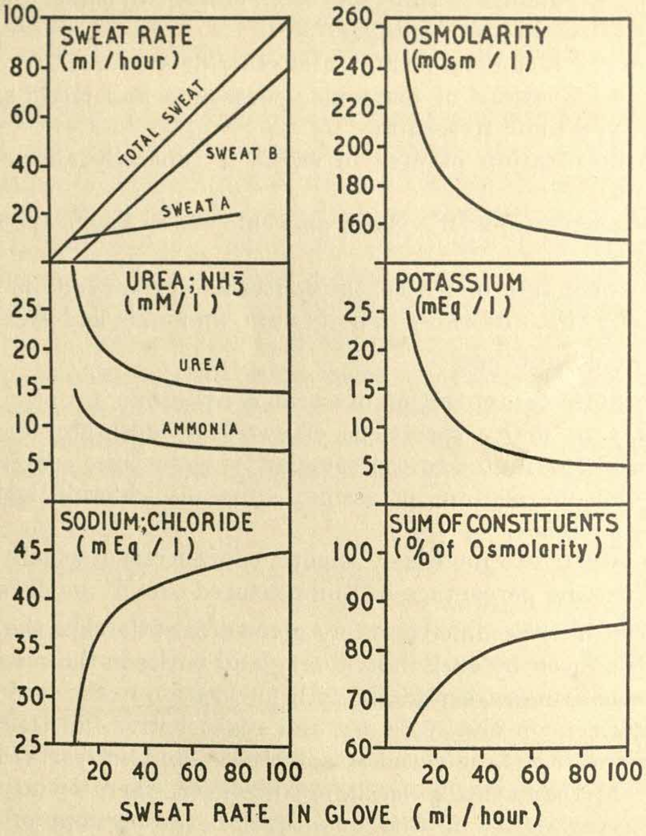

FIG. 2.—Hypothetical curves illustrating consequences of assuming two kinds of sweat, the first preponderant at low rates of sweating, the second preponderant at high rates of sweating. Ordinates, units of concentration; abscissæ, sweat rate within the glove, ml./hr. "Sweat A" assumed to have composition: Na, 25 m.equiv/l.; Cl, 25 m.equiv/l.; K, 25 m.equiv/l.; NH_3, 15 m.equiv/l.; lactic acid, 35 m.equiv/l.; urea 30 mM/l.; "osmol X" 95 mOsm/l.; and total osmolarity, 250 mOsm/l. "Sweat B" assumed to have composition: Na, m.equiv/l.; Cl, 50 m.equiv/l.; K, O m.equiv/l.; NH_3, 5 m.equiv/l.; lactic acid, 5 m.equiv/l.; urea, 10 m.equiv/l.; "osmol X" O mOsm/l.; and total osmolarity, 120 mOsm/l.

There are certain criticisms which should be mentioned in relation to our work. The sweat was collected with impermeable gloves which covered the whole forearm and the hand. It is possible, but not likely, that palmar sweat and forearm sweat are radically different. We studied three subjects who wore a forearm cuff on one arm [Lichton, 1957 *a* and *b*] and an elbow length glove on the other while working in a room at about 32° C. No consistent differences in chemistry or osmolarity were detected in the samples of sweat thus collected. The questions of secretion and reabsorption caused by an impermeable barrier would hardly be relevant to our actual observations, for our primary object was a study of total osmolarity as related to chemistry, regardless of the mechanisms of sweat production or method of collection. So far as our statistical model is concerned, no assumptions are included about diffusion, active transport, or back diffusion. A final and cogent criticism is that we are treating all subjects as members of one homogeneous population in spite of the well known principles that individual idiosyncrasies are characteristics of all quantitative measurements on sweat, and that dietary regimen can cause gross alterations of sweat chemistry. The reader is requested to bear in mind our primary questions: What are the limits of variability of the freezing point of sweat? What relation exists between freezing point and chemical composition? To answer those questions, our statistical approach appears valid. So far as any mathematical model is concerned, there can be little doubt that adequate studies on individual sweat glands will permit more realistic hypotheses than are possible by any statistical manipulation of data obtained from samples of sweat, however they may be collected, from large areas of skin. We agree with Lobitz and Mason [1948]: "Not until better methods and better tools are developed will the exact nature of the sweat gland be understood".

Acknowledgments

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INHIBITION OF BILIRUBIN CONJUGATION IN RAT LIVER SLICES BY HUMAN PREGNANCY AND NEONATAL SERUM AND STEROIDS.

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Rat liver slices conjugated bilirubin with glucuronic acid in the presence of rat sera or human sera. Sera from pregnant women and newborn infants inhibited the conjugation. Conjugation in liver slices from rabbits and monkeys was inhibited to a smaller extent. The inhibitor was heat-stable and dialyzed slowly. Conjugation in rat (but not rabbit and monkey) liver slices was inhibited by a number of steroids. Neither sera nor steroids affected the rate of conjugation of *o*-aminophenol in liver slices or the conjugation of bilirubin by broken cell preparations of liver.

The inhibitory activity of sera from newborn infants with non-hæmolytic jaundice was the same as that of infants who were not jaundiced. It is probable that the inhibitor makes only a minor contribution to the development of jaundice in the newborn infant.

Sera from several domestic animals also inhibited conjugation of bilirubin in rat liver slices.

BILIRUBIN is conjugated with glucuronic acid by liver slices [Lathe and Walker, 1957 and 1958 *b*] and suspensions [Grodsky and Carbone, 1957; Schmid, Hammaker and Axelrod, 1957]. We obtained higher rates of conjugation by rat liver slices when serum was incorporated in the incubation medium: the serum protein probably serves to retain the bilirubin in solution. It was observed that the use of human pregnancy and neonatal sera and certain animal sera reduced the rate of conjugation below that obtained with rat serum or pooled human male serum. These inhibitory effects, some of which were reproduced with steroids, have been discussed in a preliminary report [Lathe and Walker, 1958 *a*].

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METHODS

Liver slices (180–220 mg.) were incubated in 2·5 ml. Ringer-phosphate-bicarbonate solution containing 14·6 per cent (v/v) serum (0·365 ml.) and 0·0146 per cent bilirubin, in an atmosphere of 95 per cent O_2, 5 per cent CO_2. Conjugated bilirubin was estimated by the direct van den Bergh technique. Full details of the experimental procedures used for bilirubin and *o*-aminophenol conjugating systems and of the treatment of animals are given in a previous paper [Lathe and Walker, 1958 *b*]. Conjugated *o*-aminophenol was estimated by the method of Levvy and Storey [1949].

Sera.—Adult human blood was obtained by venepuncture. In newborn infants capillary blood was drawn by heel prick. Fœtal blood was obtained from the placental veins. Maternal and fœtal bloods were drawn within a few minutes of delivery. Plasma from "exchange" transfusions of newborn infants with hæmolytic disease or non-hæmolytic jaundice were provided by the pædiatricians at Queen Charlotte's Maternity Hospital. Pooled human male serum was used as a control. Controls were studied in quadruplicate, human venous specimens and animal sera in duplicate and capillary specimens singly.

Test Substances.—Most of these were dissolved in ethanol and measured into the incubation flasks, the solvent being removed by evaporation before the addition of the incubation medium. Ethanol-insoluble substances were added in aqueous solution. Œstriol (œstra-1 : 3 : 5(10)-triene-3 : 16α : 17β-triol), 3α-hydroxy-5α-pregnan-20-one, 5β-pregnane-3α : 17α : 20α-triol and 3α : 17α-dihydroxy-5β-pregnan-20-one were provided by Miss M. J. Stern. Œstradiol-17β (œstra-1 : 3 : 5(10)-triene-3 : 17β-diol), œstrone (3-hydroxy-œstra-1 : 3 : 5(10)-triene-17-one), progesterone (pregn-4-ene-3 : 20-dione), testosterone (17β-hydroxy-androstan-4-ene-3-one), pregnandiol (5β-pregnane-3α : 20α-diol) and 5β-pregnane-3β : 20β-diol were from Organon Ltd. Dr. R. W. H. Edwards provided glucuronides of 5β-androsterone (3α-hydroxy-5β-androstane-17-one), androsterone(3α-hydroxy-5α-androstane-17-one), pregnandiol and testosterone (contaminated with 5β-androsterone glucuronide). The remainder of the steroids were from the M.R.C. Steroid Reference Collection. Professor A. A. Harper made a gift of secretin; Glaxo Laboratories gave triiodothyronine and thyroacetic acid, and Roche Products gave gantrisin. Rhemannic acid was given by Professor C. Rimington. Animal sera were provided by Dr. Barbara H. Billing, Mrs. J. M. Hewitt and Professor H. D. Kay.

RESULTS

Inhibition of Bilirubin Conjugation by Pregnancy Serum.—The rate of conjugation of bilirubin by liver slices under standard conditions showed considerable variation between animals of the same species. In order to compensate for this variation the rate of conjugation in the presence of pooled male serum (the control determination) was estimated in quadruplicate for each animal. The effect of substituting other sera, or of adding test substances, is expressed as a percentage inhibition of the rate observed with pooled male serum. Tests and controls were always made with slices from the same liver. Six single adult human male sera examined in duplicate gave negligible inhibition (0, −9, 0, 14, 16, 14 per cent) of conjugation by adult male rat liver slices, when compared with rat serum.

A number of sera (3 to 8) were taken at intervals throughout one menstrual cycle from eight women. Inhibition of bilirubin conjugation by male rat liver slices varied from 0 to 25 per cent. The inhibitory effect did not appear to be related to the stage of the menstrual cycle.

In nine patients the serum showed a progressive increase of inhibition during pregnancy and a return towards normal after delivery (fig. 1). The relative inhibition by fourteen maternal sera and the corresponding fœtal sera at delivery is given in fig. 2, which also shows the fall in inhibitory power of sera in two healthy infants during the first few days of life. Five infants who developed non-hæmolytic jaundice showed a similar fall.

Comparative Studies.—Bilirubin conjugation by male and female animal livers was inhibited by sera obtained from the blood removed at exchange transfusions (Table I). Liver slices from pregnant, non-pregnant and male rats behaved similarly.

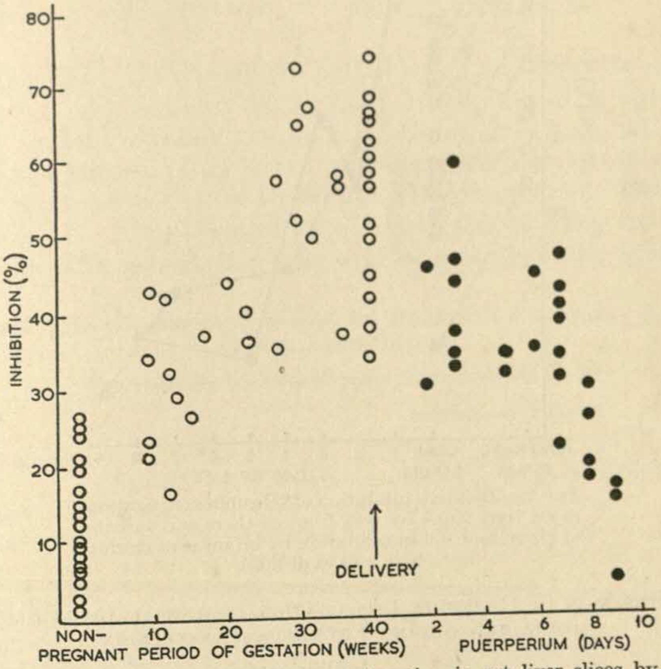

Fig. 1.—Inhibition of bilirubin conjugation in rat liver slices by human sera obtained during pregnancy and the puerperium.

The effects of the sera of various animals on conjugation by male rat liver slices are given in Table II.

Sera from three women, 30, 36 and 36 weeks pregnant, inhibited conjugation by male rabbit liver slices by 20 per cent, 26 per cent and 40 per cent respectively. Nine sera from four full-term normal babies, not jaundiced during the neonatal period, inhibited conjugation by male rat liver slices by 16 to 62 per cent. Serum from a pregnant rat did not inhibit conjugation by male rat liver slices.

Human sera were examined for their effect on conjugation by monkey liver slices. The average inhibition by eight maternal sera taken at delivery was 23 per cent (Range − 20 to + 50 per cent), and by the corresponding placental sera 14 per cent (Range − 14 to + 40 per cent). Infant sera during the first 9 days of life did not show the gradual decline in inhibition observed when rat liver was used. The range for infants 7 days old was the same as that found in placental sera.

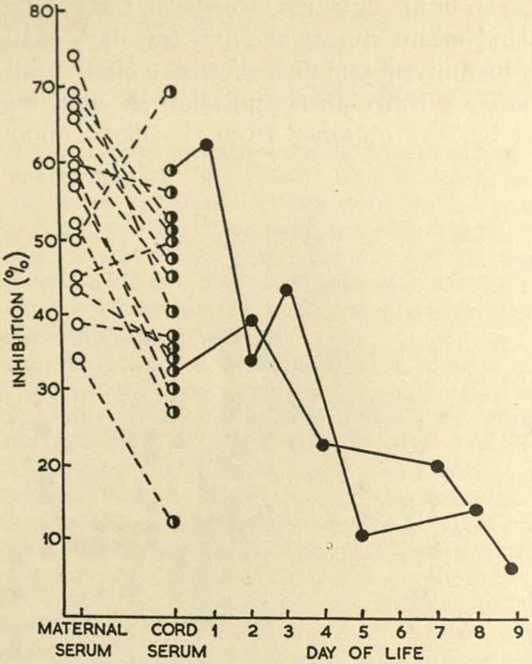

FIG. 2.—Relative inhibition of bilirubin conjugation in rat liver slices by sera from mothers and infants at birth, and fall in inhibition by infant sera during first 9 days of life.

TABLE I.—INHIBITION OF BILIRUBIN CONJUGATION IN ANIMAL LIVER SLICES BY SERA OBTAINED DURING EXCHANGE TRANSFUSION

	Inhibition per cent				
Serum	Male rat	Female rat	Male rabbit	Male monkey	Female monkey
A I	43, 41, 47, 35	42	55	10,* 39, 23, 16, −9	33
B I	24	22	..	−16*	40
C II	36	48	..	39*	36
D I	63, 72	56	..	−1*	35
D II	77	..	..	..	..
E I	16	25	..	−2*	33

I, first exchange transfusion; II second exchange transfusion.

* These determinations were made on the same liver.

Factor Responsible for Inhibition.—A number of substances were examined for ability to inhibit bilirubin conjugation by rat liver slices. Glucuronate (20 mM) caused 37 per cent inhibition. No inhibition was found with the following substances: glucose (20 mM), sulphate (2·4 mM), propylene glycol (260 mM), phenolphthalein β-glucuronide (4 μg./ml.), rhemannic acid (4 μg./ml.), histamine dihydrochloride (74 μg./ml.), acetyl-β-methyl choline

TABLE II.—INHIBITORY EFFECT OF ANIMAL SERA ON THE BILIRUBIN CONJUGATION BY MALE RAT LIVER SLICES

Each figure is the mean of duplicate estimations with serum from one animal.

Animal	Inhibition per cent	Animal	Inhibition per cent
Goat	60, 30, 11	Bull	50
Sheep	− 10, 0, − 12, 57	Steer	56, 60
Pig	40, 53	Cow	50, 53, 68, 55, 60, 41
Horse	47, 56	Cat	13, 28, 35
Rat	3, 4	Dog	65, 47, 58, 56

chloride (70 μg./ml.), L-thyroxin (20 μg./ml.), L-triiodothyronine (4 μg./ml.), insulin (0·8 mg./ml.), secretin (20 μg./ml.), nicotinic acid (4 μg./ml.), nicotinamide (4 μg./ml.), Synkavit (4 μg./ml.), Gantrisin (40 μg./ml.) pentothal (50 μg./ml.) and borneol (4 μg./ml.). Steroids, some of which had a marked effect, were examined with liver slices from several animals. The results are given in Table III. The effect of steroids was also investigated using liver slices from pregnant rats, and results were comparable with those obtained

TABLE III.—EFFECT OF STEROIDS ON BILIRUBIN CONJUGATION BY ANIMAL LIVER SLICES

The medium contained 4 μg. of steroid per ml.

Steroid	Inhibition per cent			
	Male rat	Female rat	Male rabbit	Male monkey
5α-androstane-				
3α-ol-17-one	16	11	16	7
3 : 17-dione	− 21, 4	..	28, 3	7
5β-androstane-				
3α : 17α-diol	48	45	2	..
3α : 17β-diol	56	..	13	..
3α-ol-17-one	57	57	− 2	0
3β-ol-17-one	15, 63	..	..	..
3α : 11β-diol-17-one	− 12	..	..	29
3α-ol-11 : 17-dione	6, 2, 13, 45	..	2	..
17α-ol-3-one	− 9, 44	..	..	..
17β-ol-3-one	78, 69, 82, 18, 70	70	..	..
3 : 17-dione	19, 69, 65	64, 40	5	0
Androst-4-ene-				
3β : 17β-diol	23	..	..	..
3 : 17-dione	18	..	0, 8	1
17α-ol-3-one	− 12	..	..	..
17β-ol-3-one	2, 2	7	28, 26	− 5
11β-ol-3-: 17-dione	4	..	..	0
3 : 11 : 17-trione	2	..	− 17, 7	15
Androst-5-ene-				
3β : 17β-diol	4	..	..	..
3β-ol-17-one	25, 18	− 28, − 11	9	− 5
5α-pregnane-				
3α : 20α-diol	9	..	− 19	− 20
3 : 20-dione	− 9	..	− 10, − 16	..
3α-ol-20-one	2, 21, 5	..	− 20, − 16, − 2	..

TABLE III.—(*continued*)

Steroid	Inhibition per cent			
	Male rat	Female rat	Male rabbit	Male monkey
5β-pregnane-				
3α : 20α-diol (Organon)	14, 43	..	10, −17	..
(M.R.C.)	81, 25, 50, 83, 22	59	..	−12
3α : 20β-diol	26, 63	..	12	−2
3β : 20β-diol	69	..	−19	2
3α-ol-20-one	69	..	15, −30	..
3α : 21-diol-20-one	55	..	−2	..
3β-ol-20-one	72	71	−12	..
3 : 20-dione	62	71	0	−17
3α-17α-diol-20-one	51, 59	..	6	..
3α-17α : 20α-triol	47, 47	..	4, −10	..
3α : 17α-diol-11 : 20-dione	9, −7	..	23	23, 23
3α : 17α : 21-triol-11 : 20-dione	23, 11	..	−3, −10	..
3α : 11β : 17α : 21-tetrol-20-one	−4	..	−23, −13	..
Pregn-4-ene-				
3 : 20-dione	44, 52	5, 3	12	0
11α-ol-3 : 20-dione	11	..	−10, 16	−33
11α : 21-diol-3 : 20-dione	14	..	..	..
11β : 21-diol-3 : 20-dione	15	..	10	−5
11β : 17α : 21-triol-3 : 20-dione	1	..	20	−7
17α-ol-3 : 20-dione	13	..	23	..
17α : 21-diol-3 : 20-dione	23	..	..	..
21-ol-3 : 20-dione	66, −8, 70	−38, 0	−17	−42
17α : 21-diol-3 : 11 : 20-trione	0, 7, 14	−18	−10	..
11β : 21-diol-3 : 20-dione-18-al (18→11-hemiacetal)	10	..	..	..
Pregn-5-ene-				
3β : 20α-diol	−14	..	..	..
3β : 20β-diol	−16	..	..	..
3β-ol-20-one	−22	..	..	7
3β : 21-diol-20-one	−8	..	..	..
3 : 5-*cyclo*-androstane-				
6β-ol-17-one	−7	..	11	..
Cholesterol	10	..	..	..
Œstrogens				
œstrone	2	−6	−10	−19
œstradiol	6	24	−2	−6
œstriol	0	..	..	−14
Conjugated steroids				
pregnandiol glucuronide	56, 77, 80	..	7	−5
androsterone glucuronide	34	..	..	..
ætiocholanolone glucuronide	80	..	..	..
testosterone glucuronide	−14	..	..	..
pregnenolone sulphate	14	..	..	..
Bile Acids				
cholic acid	5	..	..	..
taurocholic acid	4	..	..	..
deoxycholic acid	−4	..	..	..
lithocholic acid	17, 13, 35	..	..	..

from normal male or female rats. Pregnandiol was observed by Holton [1957] to inhibit by 65 per cent at a concentration as low as 0·4 μg./ml.

Neither inhibitory sera nor steroids had any effect on the conjugation of bilirubin by rat liver suspensions, with or without the addition of a uridinediphosphateglucuronic acid concentrate.

The effects of several inhibitory human sera and some of the most potent steroids were assayed on the *o*-aminophenol conjugating system [Levvy and Storey, 1949] in rat liver slices, but no inhibition was observed.

Preliminary experiments indicated that an inhibitor of bilirubin conjugation may be dialyzed slowly from exchange sera. A 4-day period of dialysis against Ringer-phosphate-bicarbonate solution reduced the inhibitory power of a serum from 43 to 7 per cent. After 2 days the dialysate inhibited conjugation by 23 per cent; boiling this preparation for 2 min. did not affect the inhibition. The inhibitor was largely removed by protein precipitation, and not easily extracted into organic solvents.

DISCUSSION

Human adult male sera were similar to rat sera in their enhancement of *in vitro* conjugation of bilirubin by rat liver slices. It has been shown that the rat [Lathe and Walker, 1958 *b*], like man, guinea pig [Schmid *et al.*, 1957] and dog, conjugates bilirubin with glucuronic acid. The sera of six domestic animals inhibited this conjugation (Table II).

The rise of inhibitory activity during pregnancy (fig. 1) was probably due to the accumulation of an inhibitor substance, since the activity could be dialyzed away. The lower level of activity (compared with the mother) in all but one of the fœtal sera (fig. 2) suggested that the inhibitor was of maternal origin and passed to the fœtus through the placenta.

The possibility that this phenomenon was due to competitive inhibition of "bilirubin glucuronyl transferase" by steroids (Table III) was not consistent with their failure to inhibit bilirubin conjugation in broken cell preparations of liver. Moreover, several steroids known to be conjugated with glucuronic acid in the liver, *e.g.* testosterone [Fishman and Sie, 1956] and $3\alpha:17\alpha:21$-trihydroxy-5β-pregnane-11:20-dione (tetrahydrocortisone) [Isselbacher, 1956] were not inhibitory. It is possible that the inhibitory agent exerts its effect on the permeability of the hepatic cell to bilirubin or its glucuronide. Interpretation was made difficult by the failure of rabbit and monkey liver slices to be inhibited by steroids (Table III). Pregnancy and neonatal sera reduced conjugation in these species, but not as much as in the rat.

The inhibitory steroids included a number of physiological compounds (pregnanediol, progesterone, pregnanolone, deoxycorticosterone and ætiocholanolone) but not testosterone, androsterone, cortisone, cortisol, or œstrogens. Because of the limited range of steroids available it has been necessary, in analyzing the results, to assume that 3α and β-ol, 3-one, 17α and β-ol and 17-one, which did not block inhibitory activity of the steroids when they occurred with the 5β configuration of androstane and pregnane, would not interfere when they occurred with other configurations, and with double bonds in the 4, 5 and 5, 6 positions. On this basis it may be stated that the following characteristics are each compatible with inhibitory activity: 5β-androstane, 5β-pregnane, pregn-4-ene, 3α-ol, 3β-ol, 3-one, 17α-ol, 17β-ol, 17-one, 20α-ol, 20β-ol, 20-one and 21-ol. Any one of the following characteristics prevents the inhibitory action: 5α-androstane, androst-4-ene, androst-5-ene, 5α-pregnane, pregn-5-ene, 11α-ol, 11β-ol, 11-one and a phenolic A ring.

The fact that many steroids were active at a concentration of 4 μg./ml., and pregnandiol at 0·4 μg./ml., approaching the physiological range, suggests that the unknown substance may possibly be a steroid. Among adrenal products plasma 17-OH corticosteroids behave like the inhibitor in respect of the rise in pregnancy [Bayliss, Browne, Round and Steinbeck, 1955]. The level in cord blood is less than in maternal blood [Gemzell, Robbe and Ström, 1956; Migeon, Prystowsky, Grumbach and Byron, 1956], but the inhibitor did not increase during labour (fig. 1) as do the 17-OH corticosteroids [Gemzell *et al.*, 1956]. Corticosteroids leave the infant blood more rapidly after delivery [Klein, 1954] than did the inhibitor (fig. 2). Plasma 17-ketosteroids differ from the inhibitor in having a higher concentration on the fœtal side of the placenta [Gardner, 1956], and their concentration in serum drops more rapidly after birth [Gardner and Walton, 1954]. Progesterone has been reported to be present in the umbilical vein at a similar concentration to that found in the non-pregnant female in the luteal phase of the menstrual cycle [Forbes, 1950 and 1955]. The concentration of inhibitor is much higher in the cord blood (figs. 1 and 2). Plasma values for preganediol are not available, but the drop in concentration in both maternal and infant urine immediately after birth is great [Coyle, Mitchell and Russell, 1956; Roby, Ober and Drorbaugh, 1956]. Thus the distribution of the inhibitor differs in some respects from all of these.

The possibility that the inhibitor was responsible for neonatal jaundice in the absence of hæmolytic disease has been considered. Three pieces of evidence were against this. The plasma inhibitor was inactive in broken liver cell preparations but both slices and broken cell preparations of newborn human liver showed a reduced capacity to conjugate bilirubin [Lathe and Walker, 1957 and 1958 *b*]. Secondly, no differences in the degree of inhibition of rat and monkey liver slices were shown by the sera of jaundiced infants and normal ones. Thirdly, pregnant women are not normally jaundiced, although their sera are more inhibitory than those of their newborn infants. Their freedom from jaundice is probably due to the high capacity of liver for excreting bilirubin, which exceeds the normal bilirubin load by about a hundred times. Two-thirds inhibition would still leave ample excretory capacity. Nevertheless, in the presence of the basic enzyme defect shown by Lathe and Walker [1958 *b*] the inhibitor effect might play a part in determining the height to which the bilirubin would rise. An assessment of this point is well beyond the limits of present methods, and is probably not important as it presupposes a major defect in the conjugating system. The nature of the inhibitor is being examined further.

ACKNOWLEDGMENTS

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SOME EFFECTS OF TOLBUTAMIDE ON THE PANCREATIC ISLETS OF GROWING RATS.

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Young white rats, when given tolbutamide by mouth up to their fourth month, maintain the morphology of the pancreatic islets at a juvenile stage. The alpha/beta cell ratio does not fall at puberty to the usual low value because of decreased beta-cell multiplication; yet the activity of these cells is generally increased. The persistence of many alpha cells is interpreted as compensatory to the hypoglycæmia due to tolbutamide.

INTRODUCTION

THE hypoglycæmic action of sulfonamides is believed to be due mainly to a direct stimulation of pancreatic beta-cell secretion and multiplication [Loubatières, 1957]. This has been generally confirmed [see a German

Symposium published in *Deut. Med. Wschr.* 1957, **82,** 36], while peripheral extrapancreatic actions of sulfonylhureas have also been postulated [see an American symposium published in *Ann. N.Y. Acad. Sci.* 1957, **71,** 1). These hypotheses are summarized in a recent paper by Cavallero, Malandra and Mosca [1957]. On the other hand a physiological stimulation to beta-cell reproduction takes place in the rat at about puberty. In this way the relatively high juvenile ratio of alpha/beta cells falls when the adult age is reached [Mosca, 1956]. Thus it is of interest to investigate the possible action of tolbutamide on the islet apparatus during this period of physiological change.

MATERIALS AND METHODS

White rats of both sexes were randomized for each experimental group from six different litters. The initial age and body weight were 47 days and 55–65 g. Tolbutamide ("Ipoglicone", kindly supplied by Farmitalia, Milan) was given daily to the treated animals in an amount of 100 mg./100 g. body weight, suitably mixed in the food. All control and treated animals were pair-fed on a well-balanced diet rat-cake, to which small amounts of $NaHCO_3$ were added. Water was freely allowed. At the end of the experiment, 9 hr. before their sacrifice, the animals received a subcutaneous injection of colchicine in a dose of 0·15 mg./100 g. body weight [Mosca, 1957]. This mitoses-enhancing drug facilitated the detection of which cells are fit for multiplication [Dustin, 1939; Cavallero, 1947; Cavallero and Mosca, 1953].

At necropsy, the pancreas was fixed in Helly's fluid, embedded in paraffin, thinly cut in serial sections and stained with aldehyde-fuchsin and hæmatoxylin, according to Gomori [1950] and Scott [1952]. The ratio between alpha and beta cells was estimated by counting, on the whole pancreatic surface, not less than 2000 islet cells per animal, while the total number of mitotic figures and the alpha/beta mitotic ratio were estimated on at least 4000 cells per animal. The significance of differences between groups was tested with Student's "t" test and probability "P". Each mean is the average value of all figures concerned for single animals.

RESULTS

Fig. 1 shows that the body weight of the untreated controls progressively increases with age. The tolbutamide treated animals, after the first 4 days of assuefaction to the drug, during which a loss of weight is noticeable, become quite healthy and start growing again. Thereafter no difference between the two curves is ever statistically significant. The pancreatic findings are summarized in Table I. A comparison of groups A, B and C shows that the alpha/beta cell ratio (see column "alpha cells") decreases with age and how this depends on the kind of islet cell multiplication (mitoses per 1000 cells) particularly of beta cells (see column "alpha mitoses"). Moreover the average width of islets (number of cells per islet) appears to be significantly smaller in adult than in young rats.

The tolbutamide treatment does not seem to interfere with islet morphology during the period corresponding to the usual decline of alpha/beta cell ratio. Nevertheless a tendency toward a lower beta-cell mitotic ratio appears (group D "alpha mitoses"). This is definitely evident in animals treated for a longer period who become adult (group E "alpha mitoses").

In these the total number of mitoses decreases as in the controls (mitoses per 1000 cells); but, because of the scarce beta-cell multiplication (see column "alpha mitoses"), the final alpha/beta cell ratio remains high. It seems, in other words, that tolbutamide either keeps high the mitotic rate of alpha cells, or slows down the one of beta cells. Since in these animals the total amount of mitoses per thousand cells is not increased, the latter hypothesis seems more likely. The high juvenile alpha/beta ratio and the relatively large size of the islets seem to go constantly on, as revealed by the two animals of group F. The blood sugar level of the last two animals, checked at the end of treatment, was mg. 73 and 85 per cent ml.

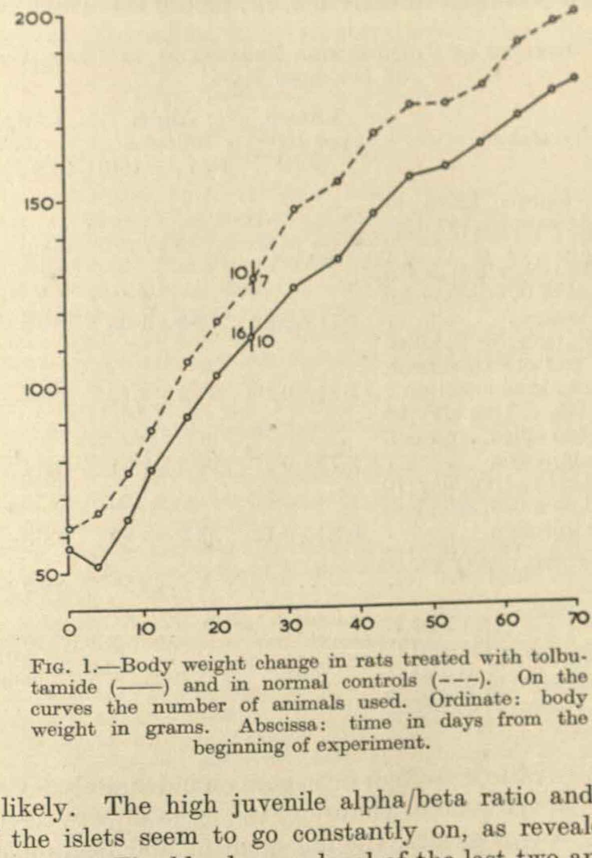

FIG. 1.—Body weight change in rats treated with tolbutamide (——) and in normal controls (---). On the curves the number of animals used. Ordinate: body weight in grams. Abscissa: time in days from the beginning of experiment.

The cytology of the islets, even in long-term treated rats, appears unaffected with regard to alpha cells, while beta cells show an enlarged cytoplasm with some degree of secretory degranulation. All nuclei are normal.

DISCUSSION

Previous experiments on a British strain of rats showed a consistent decrease of the pancreatic alpha/beta cell ratio with advancing age [Mosca, 1956]. The present findings on control animals seem to confirm the validity of this law on Italian rats. Confirmation is lacking about the action of sulfonylureas on the body weight of growing rats. Indeed, the blocking effect on body growth obtained by Ashworth and Haist [1956] with carbutamide, but not confirmed, under analogous conditions, by von Holt *et al.* [1956], did not appear under our treatment with tolbutamide, even after several weeks. With regard to islet morphology, the first findings previously described [Mosca, 1958] can now be completely supported: when the tolbutamide treatment is started in early life, *i.e.* before the usual pubertal fall of the alpha/beta cell ratio, this high ratio goes on indefinitely, even when adult age is reached and the general picture of the islets always resembles that in young animals. Cytologically, the enlargement and degranulation of beta cells and the normal preservation of alpha cells fully agree with the results of Creutzfeldt *et al.* [1957].

TABLE I.—EFFECTS OF TOLBUTAMIDE TREATMENT ON ISLET APPARATUS OF GROWING RATS

Group and No. of animals	Treatment	Mitoses per 1000 cells	Alpha mitoses ($\alpha+\beta=100$)	Alpha cells ($\alpha+\beta=100$)	No. of cells per islet
A 6	Normal controls killed at start of treatment (47 days old)	..	..	38·8 ± 2·91	81·9 ± 8·78
B 3	Normal controls killed at the same age as D, after colchicine injection . . .	2·71 ± 0·62	17·9 ± 3·06	35·3 ± 2·05	54·2 ± 9·38
C 7	Normal controls (for E) killed at the end of experiment, after colchicine injection .	1·11 ± 0·18	37·2 ± 5·13	29·8 ± 1·35	36·8 ± 3·61
D 6	Tolbutamide g. 1/kg./day/25 days, then killed, after colchicine injection . .	2·72 ± 0·27	24·5 ± 2·51	39·4 ± 1·17	57·6 ± 6·58
E 10	Tolbutamide g. 1/kg./day/70 days, then killed, after colchicine injection . .	0·93 ± 0·12	39·5 ± 5·99	38·5 ± 2·01	54·7 ± 5·24
F 2	Tolbutamide g. 1/kg./day/192 days, then killed . .	..	..	36·9 ± 1·75	51·8 ± 12·2
Statistical comparison (P<) between groups:	A and B	..	..	..	0·1
	A and C	..	..	0·015	0·001
	B and C	0·01	0·05	0·06	..
	B and D	..	..	..	..
	C and E	..	..	0·005	0·02

With regard to the mechanism of action of tolbutamide, the present findings partly counteract the hypothesis, strongly supported by Loubatières [1957], of its essential action as stimulator of beta cells. Indeed, if the stimulus due to the drug is added to the physiological stimulus due to pubertal active beta-cell multiplication, it would be expected that the mitotic ratio, especially for beta cells, would definitely increase. On the contrary neither beta cells undergo frequent mitoses nor alpha cells are decreasing in number, the final result being the conservation of the juvenile islet morphology. This seems to favour the hypothesis of an extrapancreatic site of action of the drug.

On the other hand it has been claimed that repeated insulin injections can elicit a compensatory proliferation of alpha cells [Ferner, 1952]; in this way the organism is trying to maintain its normal blood glucose level by rising the number and function of glucagon secreting cells. Even in the present animals, where the output of insulin by beta cells might be increased, as revealed by their degranulation and enlargement, it seems then likely that the persistence even in adult age of a relatively high number of alpha cells is a compensatory phenomenon to hypoglycæmia formerly enhanced by tolbutamide.

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THE INTEGRATING MOTOR PNEUMOTACHOGRAPH: A NEW INSTRUMENT FOR THE MEASUREMENT OF ENERGY EXPENDITURE BY INDIRECT CALORIMETRY.

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An instrument called the Integrating Motor Pneumotachograph (Imp) is described, designed for the continuous long-term measurement of human energy expenditure by indirect calorimetry.

The determination of energy expenditure in man can be achieved by collecting the subject's expired air for a given time and measuring its volume, and by analyzing a sample of this air so that oxygen consumption can be determined.

The Douglas bag [Douglas, 1911] has been used extensively for many years, and recently the Max Planck Institute Respirometer (M.P.I.R.), designed by Kofranyi and Michaelis [Kofranyi and Michaelis, 1940], has been increasingly employed. Both the Douglas bag and the M.P.I.R. are used only for relatively short periods because of the large bulk of the former and the limited sample storing capacity of the latter. The M.P.I.R. offers considerable respiratory resistance at ventilation rates much above 25–30 l./min. [Montoye *et al.*, 1958].

If energy expenditure for the whole of the 24 hr. is to be estimated, then a time and motion study has to be combined with the measurement of the expenditure during specific activities. This system may give rise to error if the activities of the day are very varied, and may be impossible to apply if access to the subject is limited. If an instrument could be designed which could be used for long periods without removal of the sample (1–2 hr.) and which, furthermore, was sufficiently comfortable to be worn substantially for the whole of the 24 hr., a more reliable estimation of energy expenditure could be obtained. A further requirement would be that the instrument itself should not interfere with activity or impose a limit to the maximum rate of work due either to resistance to respiratory air flows or excessive size or weight.

PRELIMINARY CONSIDERATIONS

The first question was whether the gas meter technique could be sufficiently improved to meet the above specifications. No practical attempt was made in view of the following considerations. All the energy required for the movement of bellows, the driving of the counting train, and the operation of the sampling pump has to be derived from the respiratory musculature. The energy is derived from the pressure drop across the meter, *i.e.* its resistance. The magnitude of this pressure, everything else being equal, is inversely proportional to the area of the movable walls of the bellows, and so is related to the size of the gas meter. To meet the specification the new instrument would have to be considerably larger than the M.P.I.R. A further argument against the use of a bellows type meter is the difficulty of measuring the gas temperature inside it.

It was decided to construct an instrument based not on direct volume measurement but on the measurement of instantaneous flow followed by integration with respect to time; in fact, an integrating pneumotachograph. Sampling was to be achieved by diverting a small fraction of the air passing through the flowmeter and storing it for subsequent analysis. The first step was to design a flowmeter with a linear flow/output characteristic, of low resistance but providing an output, preferably electrical, which would require little or no amplification before integration.

PRINCIPLE OF THE FLOWMETER

Fig. 1 represents a circular box fitted with an inlet tube on its cylindrical side and a re-entrant outlet tube mounted at the centre of its bottom face.

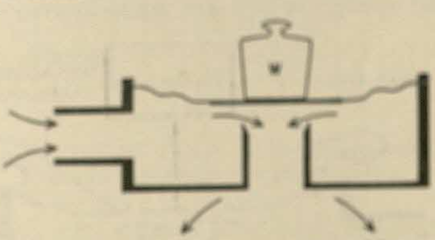

FIG. 1.—Theoretical flowmeter with weight as diaphragm restoring force.

The top of the outlet tube lies a little below the level of the top rim of the box. The top face of the flowmeter is formed by a very flexible diaphragm clamped along its circumference. The centre of the diaphragm is reinforced by a stiff, smooth plate of rather larger diameter than the outlet tube. The lower surface of the stiff disc is kept in contact with the rim of the outlet tube by a force acting along the axis of the tube. For the sake of simplicity the diaphragm restoring force is shown in the diagram as a weight (fig. 1).

As long as the diaphragm remains undeflected the outlet tube is sealed, so that no air can pass through the flowmeter. If air is blown into the inlet, the pressure in the box will rise until the force on the diaphragm is sufficient to overcome the restoring force, *i.e.* raise the weight. As soon as the diaphragm lifts, an annular gap appears between it and the top of the exit tube through which air can escape. At equilibrium the pressure inside the box must be just sufficient to raise the weight. The force required to raise the weight is independent of the height to which it is raised, so that the pressure in the box is independent of flow. At constant pressure difference the volume of air flowing through an orifice of a given type is proportional to the area so that excursion of the diaphragm is proportional to instantaneous flow.

The requirements and limitations of a practical flowmeter are as follows:

1. A restoring force which is isotonic (or very nearly so) and which is not sensitive to position.
2. A sufficiently large diaphragm area to allow a relatively small pressure (1–2 cm. H_2O) to overcome a restoring force of manageable magnitude (say 150 g.).
3. The circumference of the outlet tube has to be such that even at the maximum flow rate the area of the variable orifice is much smaller than the cross-sectional area of the tube, *i.e.* the pressure drop in the outlet tube must be small compared with that across the orifice.
4. To detect the displacement of the diaphragm a transducer must be coupled to it which is virtually frictionless, and which is linear over the range of displacement.

PRACTICAL APPLICATIONS OF THE PRINCIPLE

Fig. 2 shows a cross-section of Type A. The restoring force is applied by means of six leaf springs, which are made approximately isotonic and arranged to be already under considerable flexion when the diaphragm is undeflected. There is, however, sufficient increase in restoring force with increasing deflection to make the calibration non-linear for high flow rates. To straighten the characteristic an adjustable obstruction is placed over the end of the outlet tube. This causes a further pressure drop which increases with increasing flow. Thus, with increasing flow rate there is an increase of pressure inside the chamber which, by suitable empirical adjustment, can be made to cancel out the non-isotonicity of the springs. The weight of the diaphragm and the spring, and the moving parts of the displacement detector will, depending on the position of the flowmeter, either assist or oppose the spring restoring force, with a consequent change in calibration. Even if every effort is made to make these parts as light as possible some compensation is desirable.

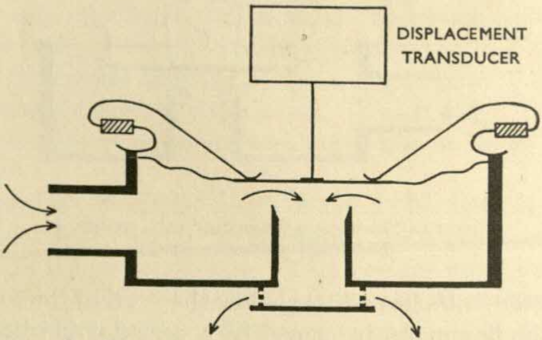

FIG. 2.—Type A—using gravity-compensated leaf springs as restoring force.

A flowmeter of this type can be made position-independent by attaching small weights to the intermediate part of the S-bend in the springs. These weights slightly reduce the force of the spring when the instrument is in the position shown in the drawing, and increase the force when inverted. The displacement of the diaphragm is detected by a micropotentiometer (see fig. 5) which is described below in greater detail.

Flowmeters of this type with full scale deflection at 500 l./min. and a back pressure at this rate of less than 1 cm. H_2O have been constructed but have only been used as laboratory instruments.

When the principle appeared to be sound, a further specification was added, as it now appeared to be possible to do away with the bulky tube conveying the air to an instrument on the back by attaching the flowmeter directly to the mask. To achieve this, both size and weight had to be reduced considerably. A simpler flowmeter was designed (fig. 3), Type B. The outlet tube had now become a coil spring which had been wound in such a way that some initial tension had been built into the spring. In the fully relaxed position the spring behaves like a tube, and a finite force (about 50 g.) was required to start to separate the turns. The top of the spring was attached to, and blocked off by, the rigid part of the diaphragm. The bottom end was attached securely around the circumference of a hole in the bottom plate of the flowmeter. When the diaphragm is deflected the turns separate and the air escapes through the spaces between the turns. The instrument can be made linear, as previously explained, by an adjustable obstruction near the outlet. The deflection is detected by a micropotentiometer.

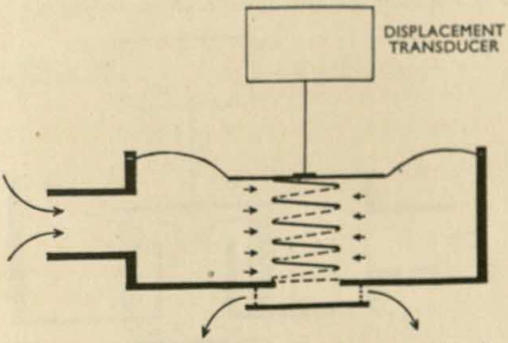

Fig. 3.—Type B—using helical spring as combined variable area orifice and restoring force.

A special version of Type B has been constructed in which the spring wire is insulated by a thin coating of a plastic. The displacement of the diaphragm is then detected by using the inductance change of the spring as it is extended to modulate the frequency of an oscillator. The change in frequency over the range of extension used is linear, and having been applied to a conventional discriminator stage gives rise to a voltage output linearly related to flow.

Type B suffers from the disadvantage that after extended use the surface of the spring is degreased so completely that water films tend to persist between adjacent turns of the spring even when they are slightly separated.

This leads to non-linearity at low flows. The effect could probably be countered by suitable surface treatment of the spring.

The version of type B in which the spring is used as an inductive transducer is valuable in applications where the pneumotachogram is required because of its high frequency response and stepless output.

Type C (fig. 4) is the design which has been adopted for the practical instrument. In construction it is very similar to Type A, except that the diaphragm restoring force is applied by three beryllium copper springs mounted inside the meter. To obtain a good approximation to isotonicity these springs have an unstretched length much less than their length when the diaphragm is undeflected. No gravity compensation was incorporated in either Type B or C because of the better ratio between restoring force and diaphragm weight which it was possible to obtain, and because it was mounted in the mask with the diaphragm in a vertical plane (fig. 12). Except in very special circumstances, it appeared unlikely that the diaphragm would be very far from vertical for any appreciable time, and that on the whole displacements from the vertical would occur equally in both directions.

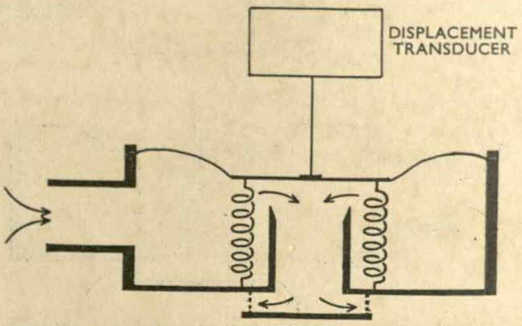

FIG. 4.—Type C—using internal phosphor bronze springs as restoring force.

The Diaphragm.—The flexible part of the diaphragm is made from ·0005 in. terylene sheet which is glued to the upper surface of a nickel-plated telephone diaphragm which forms the rigid portion. A silicone pressure-sensitive adhesive is used. The joint is reinforced by a ring made out of the same terylene sheet glued to the under surface of the rigid portion and overlapping onto the flexible portion. Neither the diaphragm nor the springs appear to corrode even after extended use. The linearizing disc is spaced from the flowmeter proper by a number of thin washers. The number of spacing washers is empirically adjusted to obtain good linearity. A thermometer is fitted into the flow meter (fig. 6).

The Micropotentiometer.—The mechanical arrangement is illustrated in fig. 5. The push rod passes through the centre of the coil wound with ·001 in. platinum iridium wire, and is constrained to move along the axis of the coil by two 1 mm. cageless ball races mounted one at each end. The slider is formed from a single strand of ·004 in. gold wire bent into the form of a small hook, the curved part of the hook making contact with the coil. The other

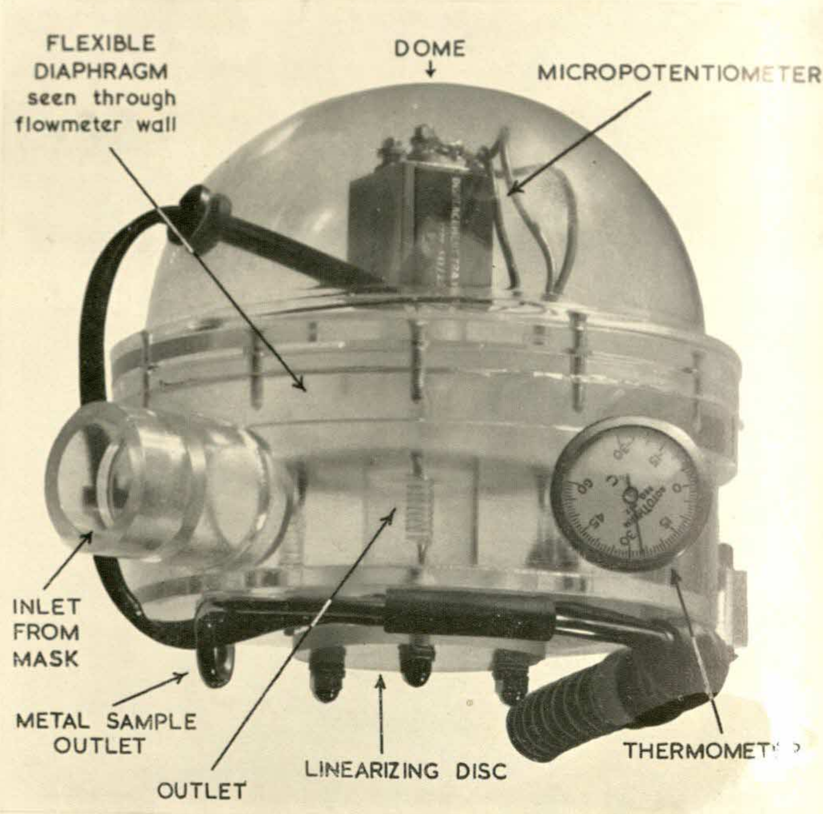

FIG. 6.—The Imp flowmeter.

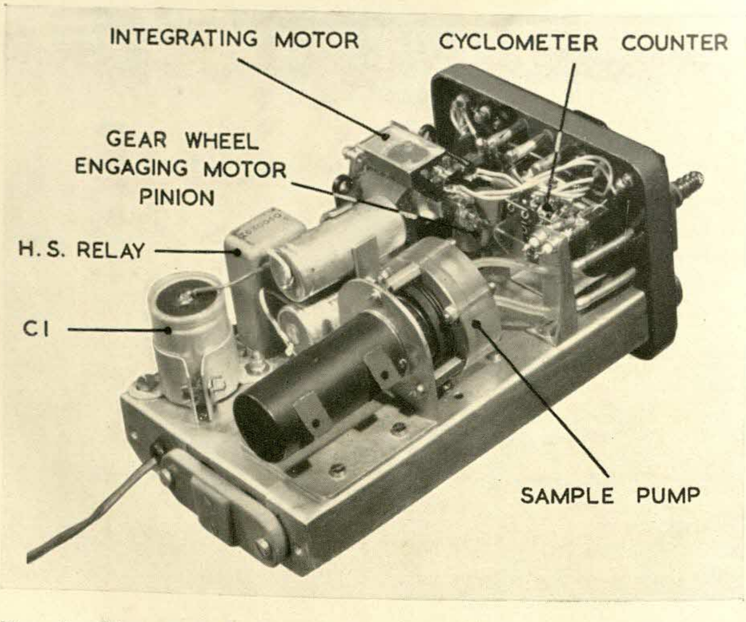

FIG. 7.—View of integrating unit showing arrangement of principal components.

end is held in a light clamp mounted on a block carried by the push rod. Major torsional movement is prevented by a fork on the block sliding loosely along a fixed rod parallel to the push rod. Minor torsional movement is of no importance because the concentric construction prevents alteration of the contact pressure, but it does help to distribute wear. Electrical connection to the slider is made by a flat helix of phosphor-bronze strip. All three connections are brought out to terminals. The potentiometer has negligible friction and has been life-tested for six million reversals without deterioration.

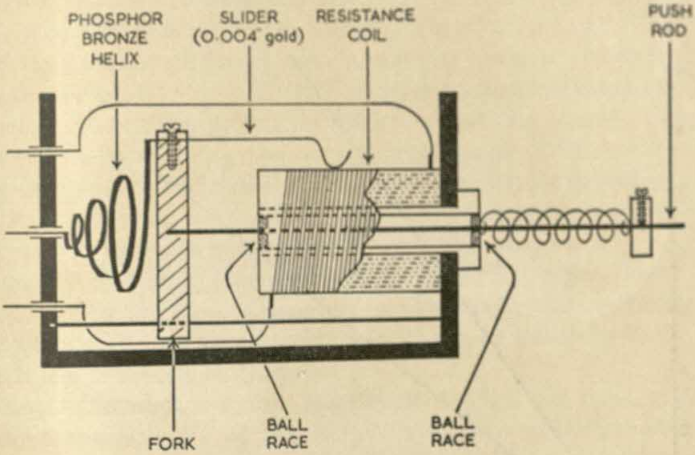

Fig. 5.—Diagrammatic cross-section of micropotentiometer.

With a coil of 200 turns the resistance is 2800 ohms. The push rod is spring-loaded against the flowmeter diaphragm by a weak coil spring fitted between the potentiometer body and a collar fitted to the rod.

If a voltage is applied to the potentiometer coil, and if the slider is adjusted to lie just off the first turn, the voltage appearing between the slider and the bottom end of the coil would be proportional to flow (fig. 8). If this voltage were integrated with respect to time, the integral would be proportional to the volume passed through the flowmeter. It would appear that for small excursions of the potentiometer the resolution, and hence the accuracy, would be very poor because of the finite thickness of the turns. For steady small flows, or for a single expiration, this is undoubtedly true. If, however, the flow is of a pulsating nature, as it is if a large number of expirations are measured consecutively, the accuracy will be much better because over- and under-deflections (*i.e.* one turn too much or one too little) will occur in such proportions as to make the effective resolution a small fraction of a turn. Nevertheless, it is recommended that flowmeters should not be used at ventilation volumes of less than one-tenth of their maximum rating.

The Integrating Unit.—The basic component of this unit is the integrating motor.* This is a permanent magnet electric motor with a special armature which revolves around a stationary iron core. The armature is therefore

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* Electro Methods Ltd., Stevenage, Herts.

light and has a low moment of inertia. The commutator is of small diameter with gold segments fed by gold wire brushes. The bearings are jewelled. Because all causes of friction have been reduced to a very small order, the motor will start at a low voltage (40 mV.). At higher voltages, up to 12 V, the speed of the motor is accurately proportional to the voltage. If such a motor is fed from a source of relatively low impedance, and is made to drive a revolution counter, the number of revolutions made in a given time is proportional to the time integral of the applied voltage.

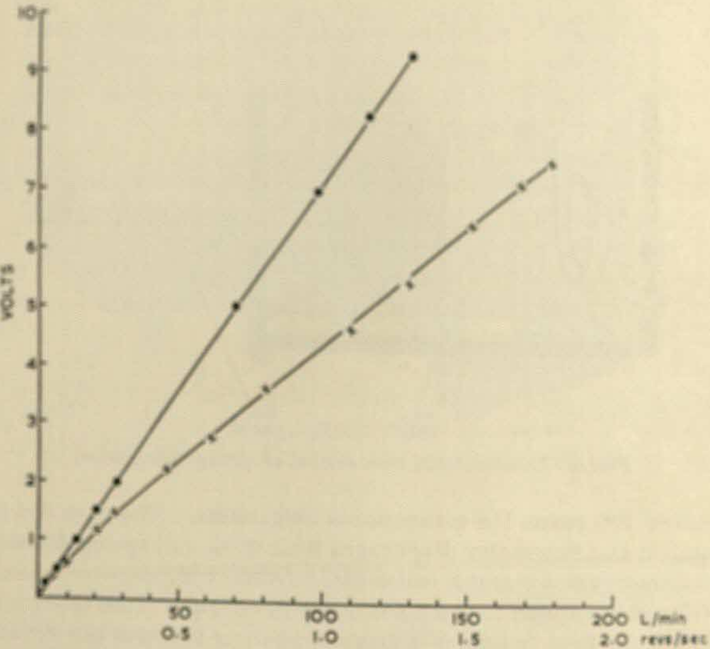

FIG. 8.—The relationship of motor speed against input voltage (●——●) and the relationship between flow rate through the flowmeter and voltage output of the micropotentiometer (X——X).

The relationship between the voltage applied to the input of the transistor impedance transformer (see below) and the speed of the motor is plotted in fig. 8. The graph is a straight line with an intercept on the voltage axis of about 50 mV. This represents the starting voltage of the motor, and is equivalent to a movement of less than 0·002 in. on the micropotentiometer. On the same figure the relationship between the rate of air flow through the flowmeter and the voltage output of the micropotentiometer is plotted. The slight non-linearity near the origin is probably due to the lack of resolution of the micropotentiometer for very small diaphragm excursions.

The speed of response of the motor is more than adequate for the rate of change encountered in respiratory airflows (from rest to correct speed in 10–20 msec.). Fig. 7 shows the general arrangement of the integrating motor and the cyclometer counter.

The output impedance of the micropotentiometer is too high and too variable to allow it to be connected directly to the motor. A simple impedance transformer using two junction transistors is used, which not only presents a low source impedance to the motor but also allows for easy electrical zeroing. The full circuit is given in fig. 9. Power is derived from eight Mallory RM 1 mercury cells which give a substantially constant voltage throughout 90 per cent of their life and only have a small temperature co-efficient. Each set of cells is normally used for 50 hr., a life which allows a fair margin of safety.

The motor drives a cyclometer counter through reduction gearing, such that with a standard flowmeter the resolution is about 0·25 l. The integrating motor and the associated circuit has a small temperature co-efficient (approximately +·08 per cent/° C.). A large measure of compensation can be applied by connecting two Brimistors * in series across the motor. They form a potentiometer network with the output impedance of the transistors; with increasing temperature the resistance of the Brimistors decreases, leading to a fall in the fraction of the total output voltage appearing across the motor. The integrator will work satisfactorily over the range 0–60° C. The lower limit is set by the characteristics of the batteries which show increasing internal resistance and loss of capacity as the temperature is further reduced. The batteries are, however, so small that they can easily be worn separately underneath the subject's clothes.

Sampling.—To obtain a fair sample of expired air over a long period, during which the activity of the subject and his ventilation rate may vary widely, the rate of sampling must at all times be proportional to the rate of air flow. It proved to be impracticable to construct a continuously working pump, largely because of its high power requirements (the total power consumption of the integrator is less than 100 mW.). A two-track commutator was fitted onto an intermediate stage of the cyclometer counter gearing. One track makes contact with a gold wire brush approximately every 1·5 l., the other every 4·5 l. Every time contact is made condenser C. 1 (fig. 9) discharges through the high speed relay (RLA). The relay contacts close momentarily and discharge condenser C. 3 through the solenoid of a single-stroke electromagnetic pump fitted with polythene sheet valves (fig. 10). The pump draws a small volume very rapidly from the flowmeter through a thin plastic tube incorporated in the lead, and pushes it into the sample container. The stroke volume of the pump is adjustable from 0·3–2·0 ml. but is usually set to 0·3–0·5 ml. The pump strokes occur at regular volume intervals, but at random times, during the expiratory cycle unless the volume of the tidal air should coincide exactly with the sampling intervals. Experience has shown that this does not occur to a significant extent; if it did it would be expected to show up in the validation. The polythene sheet valves work better when wet—it is desirable, therefore, to suck a little water into the pump before putting it into service. The two rates of sampling are selected by means of a movable link on the front of the instrument.

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* A resistance made from a semi-conductor which has a high negative temperature co-efficient.

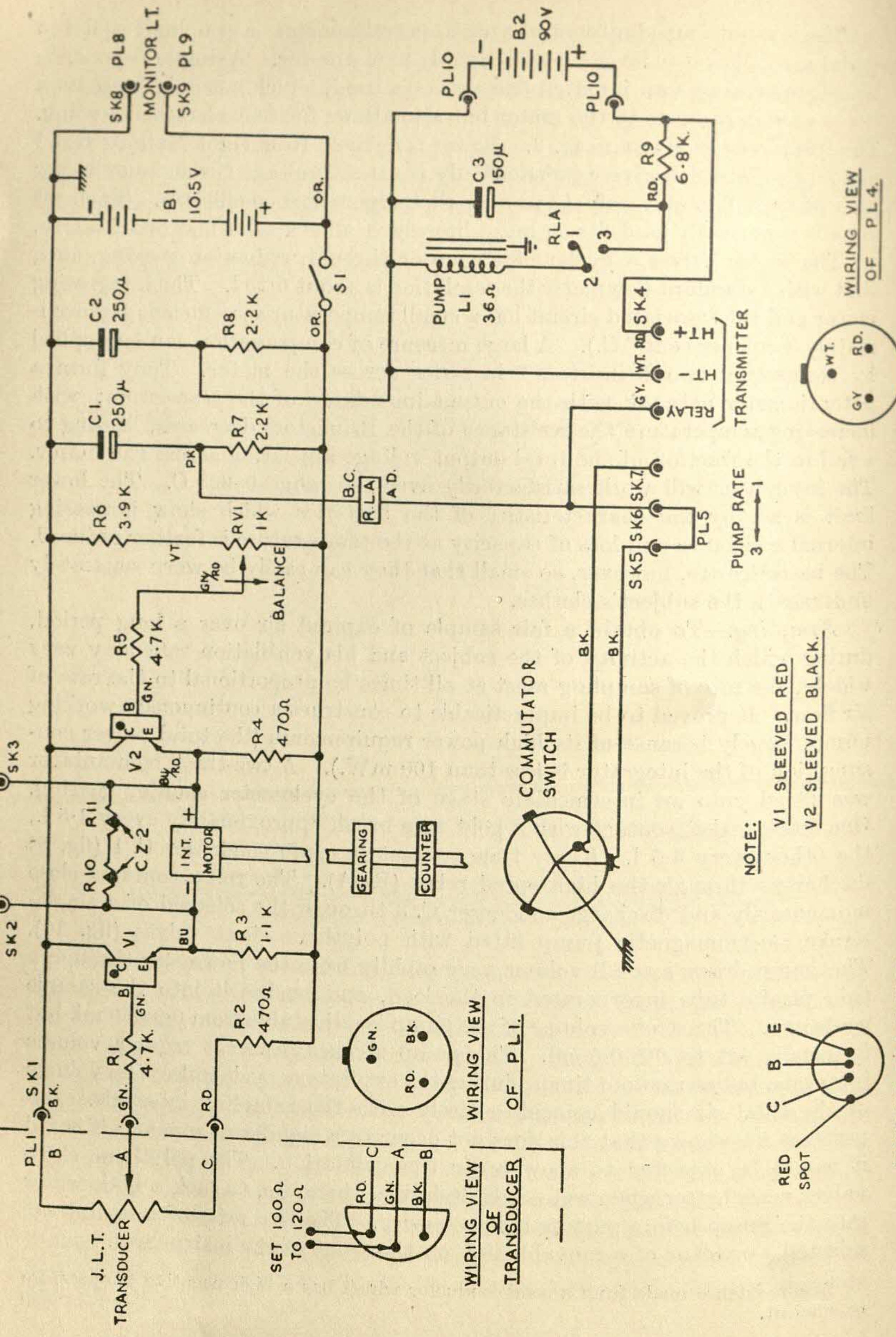

FIG. 9.—Circuit of the integrating unit.

Power for the pump only is supplied by a 90 V. layer type H.T. battery sufficient for at least 300 hr. If the strokes do occur at random points in the cycle, the integrated sample resulting from a large number of strokes will be equivalent to an average expiration.

Sample Storage.—The Imp sample had to stay unchanged in the sample bag for at least 2 hr. To keep the overall size of the instrument small it was found that the bag could not exceed a volume of about 400 ml. After considerable searching no plastic was found which would fulfil the requirements. There appears to be a relation between the flexibility of a plastic and its carbon dioxide permeability which operates in such a way that those plastics which are relatively impermeable are also stiff and have to be used in a thinner gauge to make a bag, with little resultant gain. The few plastics which proved to be an exception to this rule could not be welded or stuck satisfactorily. The problem was finally solved by making the bags out of ·008 in. laminated P.V.C. or ·010 in. butyl rubber, and suspending them inside a seamless aluminium canister filled with expired air. As the bag inflates the air surrounding it escapes through a non-return valve in the lid (fig. 11). During large scale experiments the tedium of flushing perhaps 100 tins per day becomes too great and compressed synthetic "expired" air is used, containing 3·2 per cent carbon dioxide, 16·8 per cent oxygen, and 80·0 per cent nitrogen. The reduction in diffusion gradient achieved by these means keeps the sample intact within the limits of analysis for at least 2 hr. provided that sample is continuously being pumped into the bag. The continual slight positive pressure in the tin prevents contamination of the "insulating" air with outside air. At the end of a sampling period the sample is transferred into a glass container as quickly as possible.

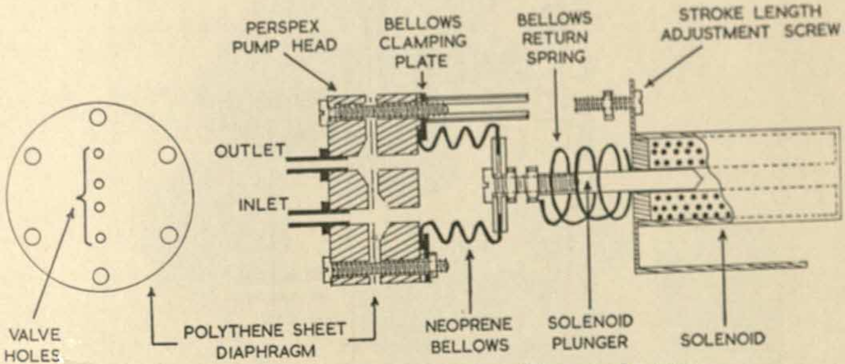

Fig. 10.—Diagrammatic cross-section of the sample pump.

The Mask.—From a subjective point of view the mask is almost the most important component of the instrument. Fortunately, the R.A.F. H-type mask provided a satisfactory basis from which to begin. In its modified form it consists of a rubber face-piece lined with chamois leather. The rubber carcass is fitted with three spring-loaded mica disc inlet valves so placed that the inspired air blows over the nose and cheeks (fig. 12). The valves are also a standard component, though normally only one is fitted to each H-mask. They are modified to have a lower opening pressure by weakening the springs. The Imp flowmeter is wired into a hole at the bottom of the mask, and does not appear to interfere with vision or the mobility of the head. The mask is held onto the face by a webbing harness passing over the back and top of the head. The masks come in three sizes, and there are facilities for adjustment both on the mask itself and on the harness. With care, most men can be fitted with a comfortable airtight mask.

Masks are tested for leaks during the fitting by occluding the hole into which the flowmeter will later be wired with a thumb or a rubber bung, and asking the subject to expire. Some expiratory effort should be required before the mask begins to leak around the edges. Virtually all leaks which do occur are around the bridge of the nose, and result in air being blown into the eyes when the subject himself can detect them very easily. All subjects are made "leak conscious" and quickly learn to tighten the appropriate straps or readjust the masks on their faces. It is believed that mask leakage is not a problem provided that the correct size of mask is chosen, that the subject is moderately co-operative, and that the small percentage of potential subjects with really "difficult" faces is rejected.

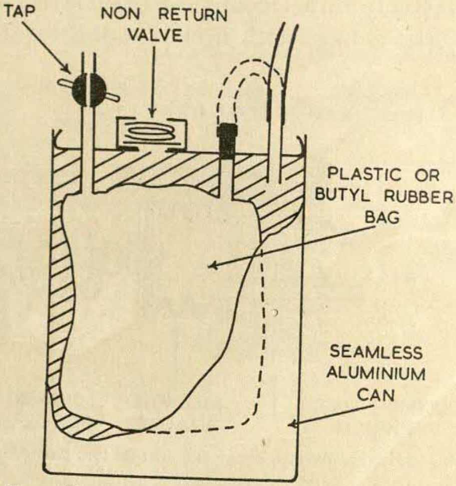

FIG. 11.—Cut-away diagram of sample tin showing the sample bag suspended inside.

There is a problem when sleeping subjects are used, it is probably most easily overcome by taping the edges of the mask to the face with non-porous adhesive strapping.

Temperature Measurement.—In a bellows gas meter the temperature is usually measured at the outlet end of the meter. If there is an appreciable temperature difference between the air at the inlet to the meter and the outside air, the expired air will cool on its passage through the meter with a consequent reduction in volume and condensation of water vapour. Experiment with an M.P.I.R. has shown that the difference between the inlet

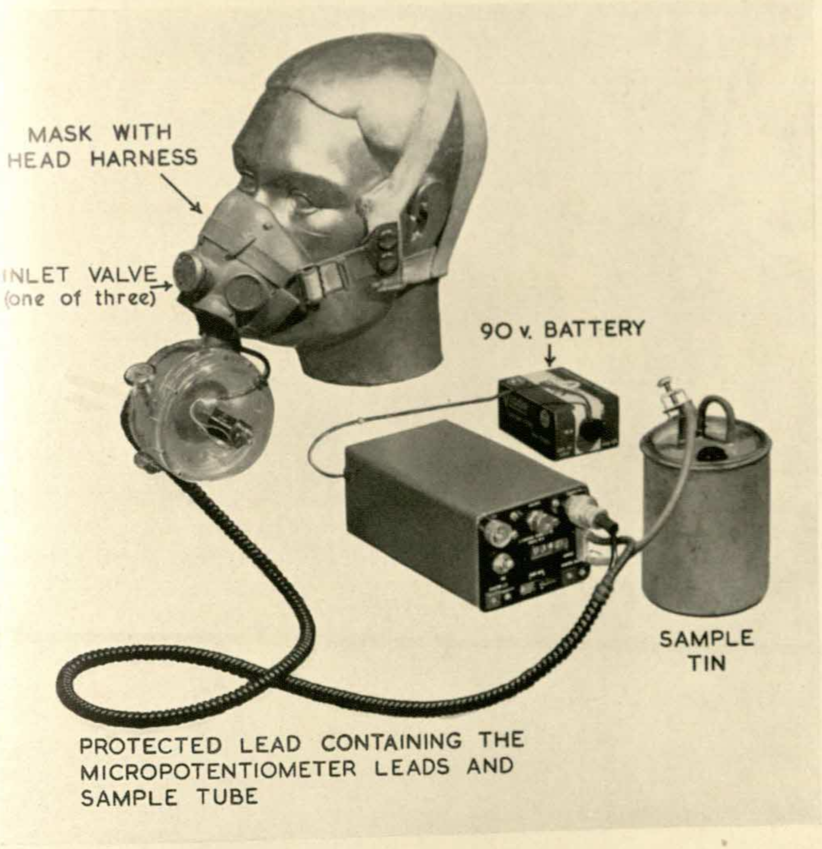

Fig. 12.—The complete instrument.

temperature and the indicated outlet temperature can easily be 10° C. (27° C. – 17° C.) at an external temperature of 10° C. and a ventilation volume of 30 l./min. Assuming that measurement is actually carried out at a temperature midway between these extremes, there will be systematic error due to this cause alone of almost 2·5 per cent in volume measurement.

TABLE I.—IMP NO. 3 CORRECTION FACTOR 2·46 (ALL VALUES AT N.T.P.) DRY

Ventilation rate (l./min.)	Corrected Imp reading (litres)	Vol. in Douglas bag (litres)	Douglas bag / Imp
5·9	418·6	419·5	1·003
24·5	423·6	423·0	0·999
26·2	429·0	428·2	0·998
28·2	426·6	424·9	0·996
34·9	429·0	429·1	1·000
45·8	423·6	422·5	0·997
47·2	426·1	423·5	0·994
52·3	429·0	427·0	0·995
54·5	429·0	429·8	1·002
55·0	421·6	422·0	1·001
71·0	420·8	418·2	0·994
Imp No. 5 Correction Factor 2·43			
6·4	211·9	213·0	1·005
18·1	418·9	417·7	0·997
35·7	422·3	423·3	1·002
46·6	421·4	419·8	0·996
46·8	421·4	420·3	0·997
51·3	421·4	420·3	0·997
62·9	423·3	419·5	0·991
81·0	418·5	417·1	0·997

TABLE II.—COMPOSITION OF IMP SAMPLE AND OF THE MIXED CONTENTS OF THE DOUGLAS BAG

Imp No.	Composition of Douglas bag sample (vol. percentage)		Composition of Imp sample (vol. percentage)		Ventilation rate (l./min.)
	O_2	CO_2	O_2	CO_2	
5	16·53	3·91	16·53	3·90	51·3
5	16·16	4·34	16·12	4·31	46·6
5	16·82	3·48	16·79	3·49	18·1
6	15·83	4·49	15·84	4·49	25·6
6	15·51	4·78	15·48	4·74	33·1

In the Imp flowmeter a bimetallic dial indicating thermometer is mounted directly in the expired air stream and close to the measuring orifice.

Performance of Standard Instrument.—The version of the instrument which has been most frequently used, and which is now in production,* consists of the units shown in fig. 12. The flowmeter is designed to measure ventilation rates in the range 6–80 l./min. Accuracy does not drop seriously until these exceed 100 l./min. The weight, including batteries and sample tin, is about 3 kg. The integrating unit, sample tin and battery are normally accommodated in a small haversack which can be worn either on the back or on the chest. All parts of the instrument can be dropped on to a hard floor from a height of 3 ft. with no more than superficial damage.

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* J. Langham Thompson Ltd., Bushey Heath, Herts.

Validation.—To validate the instrument the flowmeter was enclosed in a box in such a way that the inlet of the flowmeter could be connected to a mask via a piece of corrugated tubing while the outlet of the box communicated with a 500 l. Douglas bag. In this way all the air which passed through the flowmeter could be collected in the Douglas bag. Arrangements were made to bring the sample outlet from the flowmeter through the walls of the box.

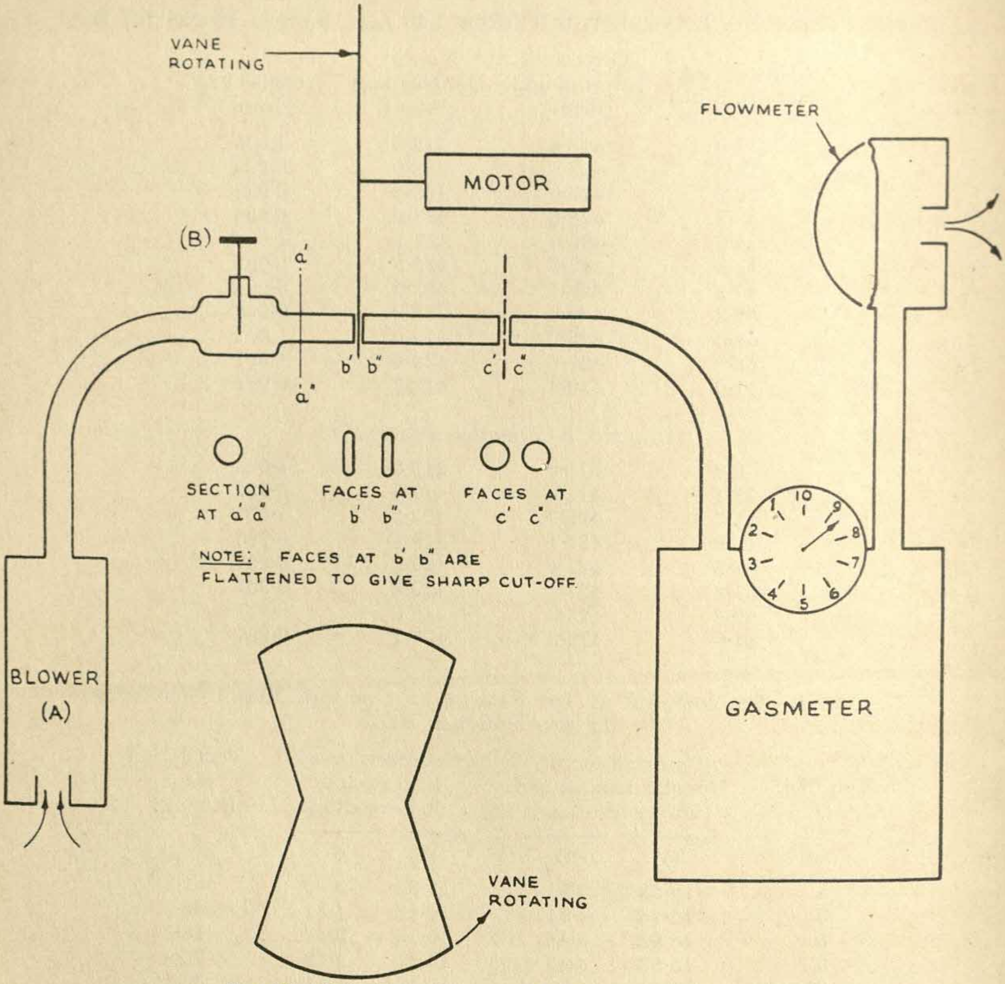

Fig. 13.—Schematic diagram of calibration equipment.

A subject wore the mask, and walked or ran on a treadmill at various speeds to produce in separate experiments ventilation rates ranging from 5·9 to 81·0 l./min.

Two series of tests were carried out, the results are shown in Tables I and II. In the first series only the volume-measuring part was checked. Two instruments were used. It can be seen that the ratio of the volume collected in the Douglas bag to that registered by the Imp differed from unity by less than 1 per cent for the whole range of ventilation rates.

Table II shows a further series of results in which the composition of the Imp sample was compared with the mixed contents of the Douglas bag or bags. The maximum difference in composition between the two samples is 0·04 vol. per cent for both oxygen and carbon dioxide.

Calibration.—The apparatus shown diagrammatically in fig. 13 is used for normal calibration. The vane is set to interrupt the flow at some convenient rate (say 20 times/min.). The "tidal air" is set by adjustment of the tap. One hundred litres are blown through the system at various tidal airs, and a calibration factor is calculated for each tidal air. Small adjustments of the linearizing disc or the micropotentiometer return spring can be used to correct minor deviations of the factor at the high and low flows respectively. The flowmeters are normally adjusted to deviate by not more than 3 per cent from the quoted factor at any flow rate within the range.

DISCUSSION

The instrument has been used for a total of more than 12,000 man-hours of measurement. It has commonly been worn for 20 hr./day for 14 days on end without any difficulty. It has proved possible to continue measurement throughout any military activity, however violent, and without serious damage to the instrument. It has always been possible to repair an instrument in the field within an hour or two of being damaged.

It does appear, therefore, that the Imp has met the requirements of portability, comfort and ability to carry out long-term experiments without sacrifice in accuracy.

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THE MATERNAL-FETAL NITROGEN EXCHANGE IN THE GOAT.*

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Determinations have been made of the amino, urea and plasma protein nitrogen of the arterial and uterine venous bloods of pregnant and non-pregnant goats and of the fetal bloods of the umbilical artery and vein respectively. The results demonstrate that in pregnant animals 67 days post insemination and beyond, amino nitrogen is lost by the maternal blood and gained by the fetal blood as they pass respectively through the uterus and placenta. Urea is lost by the fetal blood in the placenta in fetuses 90 days gestation age and older, but a regular rise in its concentration in the maternal blood perfusing the uterus was not demonstrated. No evidence was obtained to support the view that maternal plasma protein nitrogen is utilized in the growth of the tissues of the uterus or fetus during gestation.

THE experiments reported here were carried out in an effort to learn something about the sources of the nitrogen utilized in the syntheses of protein that occurs in the uterus, the placenta and the fetus as they grow during gestation. The identification of the source is of general interest, for the nitrogen so utilized may be derived from the same fraction or fractions of the blood that normally provide for the needs of the other body tissues. As the amino acids of the plasma have been assumed to provide the replacement nitrogen of the body tissues [Tarver, 1954], primary attention was focused on the changes in their concentration in the blood entering and leaving the uterus and placenta at selected stages in gestation; the concentrations of the plasma proteins and blood urea were also determined.

Because of its size the pregnant goat is especially suited to such a study for sufficient blood can be obtained for the analyses from both the maternal and fetal vessels after the 60th day of gestation. A further point of interest is the fact that the placenta of the goat is of the syndesmochorial type [Grosser, 1909], *i.e.*, the endothelial wall of the maternal capillary and a covering layer of connective tissue are interposed between the fetal chorion and the maternal blood—a relationship similar to that between the maternal blood and a representative maternal tissue cell, though it differs from the condition in man. In man and the primates, the fetal chorion is bathed by the maternal blood [Grosser, 1909].

METHODS AND MATERIALS

Blood samples were obtained from pregnant and non-pregnant goats; the breeding dates of the goats were known. All animals weighed between 20 and 60 kg. at the time of the experiment and appeared to be in good health. They were starved for

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† Toscanini Fellow.

three days before the experiment and anesthetized with intravenous chloralose (7 ml./kg. of a solution containing NaCl 5·8 g., $NaHCO_3$ 4·0 g. and chloralose 5 g. in a litre of distilled water); 100 i.u. of heparin (Liquamine) per kg. were given intravenously every 30 min.

The maternal blood samples were collected simultaneously from the femoral or carotid artery and from a large uterine vein at a rate of 1 ml./min. Thirty to 90 min. later the uterus and fetal membranes were opened, the umbilical cord delivered and samples drawn from the umbilical vein and artery simultaneously or in that order. The syringes into which the bloods were drawn were moistened with mineral oil and with a saturated solution of NaF in heparin.

Immediately after the samples were drawn the hematocrit value of each was determined by centrifugation of a portion in a Wintrobe tube for 30 min. at 3000 rev./min. A portion of the plasma so separated was used for the determination of its protein concentration with an Abbe refractometer [Hallmann, 1950]. From this value the protein nitrogen content was calculated on the assumption that nitrogen represented 16 per cent of the weight. The sensitivity and reproducibility of this method was *c.* ±16 mg. of protein nitrogen per 100 ml. of plasma.

The free amino-nitrogen of the blood was determined by the method of Hamilton and Van Slyke [1943]. The difference between duplicates was 0·10 ± 0·09 mg. of amino nitrogen. The amino nitrogen content of the anticoagulant used was 0·8 mg./100 ml.; neither this amount nor the dilution effect could alter significantly the results obtained, for the anticoagulant was so diluted in the syringes by the blood drawn that its final concentration was 1/500.

The urea nitrogen of the whole blood was determined by Van Slyke's gasometric method [1927]. The difference between duplicates was 0·2 mg. with a standard deviation of ±0·2. The water content of 1 ml. of each blood sample was determined by drying to constant weight at 105° C. in order that the urea concentration of the blood might be expressed per kg. of water.

The measurements of uterine blood flow were carried out by Metcalfe, Huckabee, Prystowsky and Barron using the 4-amino-antipyrine method they described earlier [1957].

Our ultimate goal in this research is to establish the nitrogen balance between the mother and fetus, but for technical reasons we are still short of this; so far we have not succeeded in obtaining representative samples and blood flow measurement simultaneously across the uterus and the fetus or fetuses a *conditio sine qua non* for the establishment of a balance. Because of our failure to achieve this degree of technical excellence the data obtained on the concentrations of amino acid, urea and protein nitrogen in a single experiment cannot be justifiably directly compared.

RESULTS

Amino Nitrogen.—Comparisons were made of the amino nitrogen concentrations in the bloods from the uterine artery and vein respectively of five non-pregnant goats. The results presented in Table I indicate that the concentrations—virtually the same in both vessels—ranged between 4·3 and 6·5 mg. per 100 ml.

Similar comparisons were made with bloods from twelve goats at stages in gestation ranging from the 46th to 134th day following insemination. The amino nitrogen levels (Table II) in the blood of the uterine artery ranged between 4·9 and 6·6 mg./100 ml. The concentration in the uterine vein became lower than that of the artery in does 67 days after insemination. The differences in the bloods of this group of does ranged from 0·1 to

0·8 mg./100 ml.—the vein having the lower concentration. In the samples drawn from the two does 46 days after insemination, the amino nitrogen concentration was lower in the uterine artery by 0·3 and 0·1 mg./100 ml. respectively.

TABLE I.—AMINO NITROGEN CONCENTRATION IN BLOOD OF NON-PREGNANT GOATS (MG./100 ML.)

	Source of Blood		Difference in Concentration
	Artery	Uterine vein	
	5·5	5·5	0·0
	5·6	5·3	−0·3
	4·3	4·3	0·0
	6·3	6·3	0·0
	6·4	6·5	+0·1
Mean	5·6	5·6	0·0
s	0·75	0·77	0·15
$s_{\bar{x}}$	0·38	0·39	0·08
s%	13·4	14·1	. .

Abbreviations used here and in following tables:

s =standard deviation.

s_x =standard deviation of the mean.

s% =standard deviation in per cent of mean.

t = t value of the difference between the results found and 0.

P_t =probability value of the t value.

TABLE II.—AMINO NITROGEN CONCENTRATIONS IN MATERNAL AND FETAL BLOODS OF GOATS (MG./100 ML.)

Gestation age-days	No. of fetuses	Uterine		Maternal A-V	Umbilical		Fetal A-V
		Artery	Vein		Artery	Vein	
46	1	5·9	6·2	0·3	. .	. .	. .
46	1	6·2	6·3	0·1	. .	. .	. .
67	3	6·6	5·8	−0·8	11·5	11·7	+0·2
77	2	5·8	5·2	−0·6	9·9	10·1	+0·2
					10·1	10·7	+0·6
90	2	5·2	5·2	0·0	12·3	12·6	+0·3
91	2	5·5	5·1	−0·4	10·4	10·3	−0·1
					11·8	12·1	+0·3
99	2	5·0	4·6	−0·4	9·8	11·7	+1·9
					8·9	9·4	+0·5
105	2	4·9	4·5	−0·4	10·1	10·6	+0·5
					11·5	12·7	+1·2
108	2	5·0	4·5	−0·5	11·4	11·7	+0·3
117	2	6·2	5·4	−0·8	. .	. .	. .
118	2	5·1	4·6	−0·5	. .	. .	. .
134	3	6·1	6·0	−0·1	15·4	15·9	+0·5
Mean	. .	5·6	5·3	−0·3	11·1	11·6	+0·5
s	. .	0·6	0·7	0·3	1·7	1·7	0·5
$s_{\bar{x}}$	. .	0·17	0·19	0·11	0·49	0·49	0·15
s%	. .	10·3	12·7	. .	15·3	14·5	. .
t	. .	. .	. .	3·25	. .	. .	3·45
P_t	. .	. .	. .	< 0·01	. .	. .	< 0·01

The concentration of the amino nitrogen in the umbilical artery, varied between 8·9 and 12·3 mg./100 ml. in the twelve fetuses of 67–134 days gestation age and between 9·4 and 12·7 in the blood of the corresponding umbilical vein. In all but one, the concentration was higher in the umbilical vein, the amounts ranging between 0·2 and 1·9 mg. In the single case in which the concentration was higher in the umbilical artery the difference was only 0·1 mg./100 ml. of blood.

Urea Nitrogen.—The concentrations of urea nitrogen were determined on samples of blood drawn from the uterine artery and vein respectively of the same five non-pregnant does that provided the blood for the amino nitrogen determinations. The concentrations (Table III) ranged from 8·7 to 20·6 mg. per cent with no regular difference between the concentrations in the two vessels.

TABLE III.—UREA AND PROTEIN NITROGEN CONCENTRATIONS IN THE UTERINE CIRCULATION OF NON-PREGNANT GOATS

	Urea mg./100 ml. of blood		Difference	Protein mg./100 ml. of plasma		Difference
	Arterial	Uterine vein		Arterial	Uterine vein	
	18·0	18·0	0·0	1·01	1·01	0·00
	19·2	19·4	+0·2	0·88	0·93	+0·05
	20·6	20·2	−0·4	0·80	0·78	−0·02
	8·9	9·0	+0·1	1·14	1·18	+0·04
	8·7	8·7	0·0	1·09	1·07	−0·02
Mean	15·1	15·1	0·0	0·98	0·99	+0·01
s	5·7	5·6	0·2	0·14	0·15	0·03
$s_{\bar{x}}$	2·6	2·5	0·1	0·06	0·07	0·01
$s\%$	37·8	37·4	..	14·5	15·1	..
t	..	..	0·10	..	..	0·67
P_t	..	..	> 0·90	..	..	0·6 – 0·5

In the eight pregnant does studied, the urea nitrogen concentration of the blood of the uterine artery ranged from 15·6 to 33·5 mg. per cent and in the uterine vein from 15·5 to 33·9. No regular difference was found between the concentrations of the vein and the artery (Table IV).

Comparisons were made of the concentrations of urea nitrogen from the bloods of the umbilical artery and vein respectively of six fetuses whose gestation ages ranged between 90 and 134 days. The concentrations in the umbilical artery varied from 18·3 to 38·7 mg. per cent; those of the umbilical vein from 16·8 to 38·1. The arterial concentration was always the higher by an amount that varied from 0·1 to 1·5 mg. per cent.

Expressed in mg. per kg. of water the urea nitrogen concentrations of the maternal blood ranged between 192 and 416 (mean 253) and in the fetal blood from 209 to 457 (mean 287). The concentration was higher in the fetal blood in each of the six cases available for comparison by an amount that varied from 5 to 51 mg./kg. and averaged 25 ± 7.

Protein Nitrogen.—The concentration of the protein nitrogen of the plasmas of arterial and uterine venous blood was determined on five non-pregnant and nine pregnant goats and the plasmas of the umbilical artery and umbilical vein of the ten fetuses. The results for the non-pregnant animals (Table III) indicate that the values ranged from 0·88 to 1·14 g. per cent in the arterial plasma and from 0·78 to 1·18 in the uterine venous and the absence of a significant A-V difference. In the plasmas of the pregnant goats the protein nitrogen concentration varied between 0·85 and 1·15 g. per cent in the arterial and 0·85–1·14 in the uterine venous with no statistically significant difference between the corresponding arterial and venous concentrations (Table V).

TABLE IV.—UREA NITROGEN IN THE MATERNAL AND FETAL BLOODS OF GOATS (MG./100 ML.)

Gestation age-days	No. of fetuses	Uterine		Difference	Umbilical		Difference
		Artery	Vein		Artery	Vein	
46	1	18·6	18·4	−0·2	..	..	..
46	1	20·5	20·1	−0·4	..	..	..
90	2	16·5	17·0	+0·5	18·9	18·8	−0·1
99	2	18·3	18·4	+0·1	..	..	..
105	2	15·6	15·5	−0·1	18·3	16·8	−1·5
118	2	21·5	20·8	−0·7	22·5	22·1	−0·4
133	2	19·8	19·6	−0·2	24·3	23·4	−0·9
		20·5	20·6	+0·1	23·3	23·0	−0·3
134	3	33·5	33·9	+0·4	38·7	38·1	−0·6
Mean	..	20·5	20·4	−0·1	24·3	23·7	−0·6
s	..	5·2	5·3	0·4	7·4	7·5	0·5
$s_{\bar{x}}$	..	1·7	1·8	0·1	3·0	3·1	0·2
s%	..	25·4	26·0	..	30·6	31·7	..
t	..	..	..	0·44	..	..	3·07
P_t	..	..	..	0·7 − 0·6	..	..	0·02 − 0·05

TABLE V.—PROTEIN NITROGEN IN PLASMAS OF MATERNAL AND FETAL GOATS (G./100 ML.)

Gestation age-days	No. of fetuses	Uterine		Difference	Umbilical		Difference
		Artery	Vein		Artery	Vein	
46	1	1·02	1·09	+0·07	..	..	..
46	1	1·06	1·06	0·00	..	..	..
90	2	1·07	1·04	−0·03	0·48	0·48	0·00
99	2	1·14	1·14	0·00	0·77	0·67	−0·10
					0·54	0·58	+0·04
105	2	1·08	1·12	+0·04	0·64	0·64	0·00
					0·58	0·56	−0·02
108	2	0·85	0·85	0·00	0·64	0·63	−0·01
118	2	1·02	1·04	+0·02	0·64	0·64	0·00
133	2	1·12	1·12	0·00	0·74	0·74	0·00
					0·67	0·67	0·00
134	3	1·15	1·14	−0·01	0·69	0·69	0·00
Mean	..	1·06	1·07	+0·01	0·64	0·63	−0·01
s	..	0·09	0·09	0·03	0·09	0·07	0·04
$s_{\bar{x}}$	..	0·03	0·03	0·01	0·03	0·02	0·01
s%	..	8·58	8·50	..	13·8	11·6	..
t	..	..	..	1·02	..	..	0·80
P_t	..	..	..	0·4 − 0·3	..	..	0·5 − 0·4

The concentration in the fetal plasmas was lower than that of the maternal. In the umbilical artery the plasma protein nitrogen level varied between 0·48 and 0·77 g.; in the umbilical vein between 0·48 and 0·74. Here again there was no statistically significant difference between the concentrations in the artery and vein.

DISCUSSION

To the question—which sources in the maternal blood provide the nitrogen incorporated in the tissues of the uterus, the placenta and fetus during gestation?—the data presented above appear to provide a partial answer; for they show that 67 days after insemination amino nitrogen is lost from the maternal blood as it passes through the uterus and is taken up by the fetal blood in the placenta in statistically significant amounts. Though they do not exclude the possibility that protein nitrogen is transferred from the blood of the mother to that of the fetus, they provide no support for it.

That nitrogen derived from plasma protein is used to a very limited extent —if at all—in the growth of the fetal goat and the associated membranes is not surprising. For the chorion is separated from the maternal blood by the wall of the maternal capillary and a connective tissue layer. Only the protein leaking from the maternal capillaries into the extracellular fluid would be available for absorption by the chorion and so to the fetus. Further evidence that proteins do not cross the syndesmochorial placenta is provided (1) by the absence of antibodies in the blood of fetuses having that type of placenta [Orcutt and Howe, 1922; Hansen and Phillips, 1947; Aschaffenburg *et al.*, 1949], (2) the relatively low concentration of albumin in the fetal plasma [Barboriak *et al.*, 1958], and (3) the high concentration of fetuin in the fetal plasma and its absence in the maternal blood [Pedersen, 1948]. If the placenta were permeable to protein, fetuin with its low molecular weight of 35,000 [Deutsch, 1953] might be expected to cross its barrier.

The evidence that the principal source of the nitrogen incorporated into the uterus, fetus and membranes are the free amino acids in the blood accords with observations on other organs with a rapid synthesis of protein, *i.e.* the oviduct of the fowl and the mammary gland; for amino acids are known to be utilized in the synthesis of ovalbumin [Steinberg and Anfinsen, 1952] and the casein of milk [Barry, 1952; Campbell and Work, 1952].

The demonstration by Christensen and Streicher [1948] and De Bella [1958]—confirmed by the data presented above—that the amino nitrogen concentration of the fetal plasma is higher than the maternal suggests that amino acids are actively transferred across the placental barrier. This suggestion now gains support from the demonstration that the concentration of amino nitrogen falls in the maternal blood in the uterus and rises in the fetal blood perfusing the placenta.

In some experiments the uterine blood flow was estimated by the 4-amino-antipyrine method [1957] and the A-V difference across the uterus in amino acid nitrogen was determined at the same time. In these the rate at which amino nitrogen was lost from the maternal blood can be calculated. When the flow was not measured, the rate of loss has been estimated by assuming a rate of flow using data of Metcalfe, Huckabee, Prystowsky and Barron [1957] for goats at similar stages in gestation. The flow rates observed or estimated, together with the A-V difference in the amino nitrogen concentration of the maternal blood and the estimated loss per day are presented in Table VI. So arranged the data indicate a daily loss of amino nitrogen from the maternal blood in the uterus that ranges from 2·8 to 14·5 g. per day.

These estimates of the amino nitrogen lost lead to the question: Is the amount lost sufficient to provide all the nitrogen required for growth in the uterus, fetus and placenta? An answer to this has been attempted by comparing the estimated daily nitrogen requirements with the amounts that appear to be given up in the uterus by the maternal blood.

TABLE VI

Gestation age-days	No. of fetuses	Uterine blood flow cc./min.	A-V difference amino N. mg./%/ml.	gm. amino N. lost per day	gm. N. required per day *
67	3	$245^{(d)}$	0·8	2·8	1·4
77	2	$610^{(d)}$	0·6	5·3	1·8
91	2	$730^{(d)}$	0·4	4·2	2·5
99	2	$900^{(d)}$	0·4	5·2	2·8
105	2	$800^{(e)}$	0·4	4·4	3·2
108	2	$890^{(e)}$	0·5	6·4	3·4
117	2	$1260^{(e)}$	0·8	14·5	3·7
118	3	$1340^{(e)}$	0·5	9·7	3·8
134	3	$2500^{(e)}$	0·1	3·6	4·5

* Theoretical value determined from fig. 3.
(d) = determined.
(e) = estimated from data of Metcalfe, Huckabee, Prystowsky and Barron [1957].

The daily nitrogen requirements of the growing tissues were estimated in the following way: using unpublished data of Metcalfe, Huckabee and Prystowsky, and Barron, the logarithms of the weights of the total tissues—uterus and its contents—of goats having singlets, twins and triplets respectively, were plotted against gestation age in days—see fig. 1. Representative curves were then drawn for each of these three classes. For each curve a mate was then drawn by plotting gestation age against the actual tissue weight. Similarly the logarithms of the weights of singlets and the average weights of twins were plotted against fetal age (fig. 2). A representative curve was then drawn through the points and the values on it used to plot fetal weight against age.

From the growth curves so obtained the daily nitrogen requirements for the total tissues of each group and for the fetus alone, have been estimated, assuming (1) that the protein nitrogen content of the total tissues—uterus, fetus and placenta—is 3·2 per cent of their weight (20 per cent protein), and (2) that the protein nitrogen of the fetus increases regularly from about 1·6 to 3·2 per cent of its weight between the 40th and 140th day of pregnancy. That is, the protein content increases from 10 to 20 per cent of the total weight in that interval.

These daily nitrogen requirements are presented graphically in fig. 3. The curves indicate—as is to be expected—that requirements increase with the number of fetuses, though the requirement for the individual fetus appears to be the same whether it is a singlet, twin or triplet. They further indicate that the requirement begins to be appreciable about the 35th day of gestation and increases steadily thereafter.

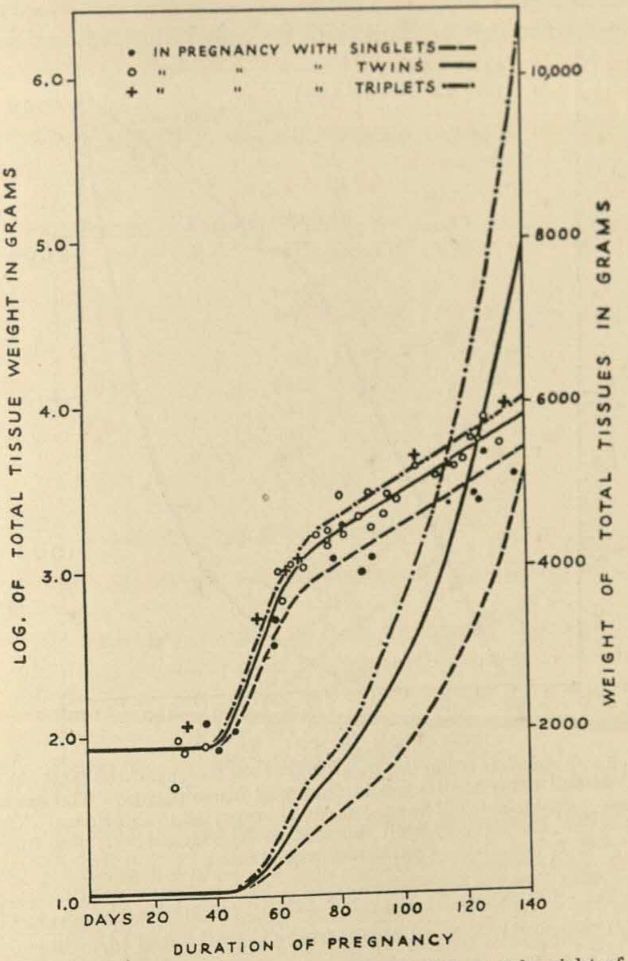

Fig. 1.—A graph relating the logarithm of the total weight of the tissue in the uterus—fetal and maternal—with the days following post insemination. For further explanation see text.

From these curves the nitrogen required for the increase in tissue mass of the uterus and its contents during the day of gestation on which the animal was studied has been estimated for each of the cases listed in Table VI. With the exception of the animal 134 days gestation age, the amount of amino nitrogen lost from the maternal blood passing through the uterus appears to be substantially higher than the nitrogen incorporated in the new tissue developed in the same interval. Similarly, provided that the umbilical blood flow is—as suggested by the A-V oxygen and CO_2 differences reported by Barron and Meschia [1954]—equal to half or more of the maternal, the daily nitrogen requirements of the fetus can be met by the amino-nitrogen taken up from the placenta.

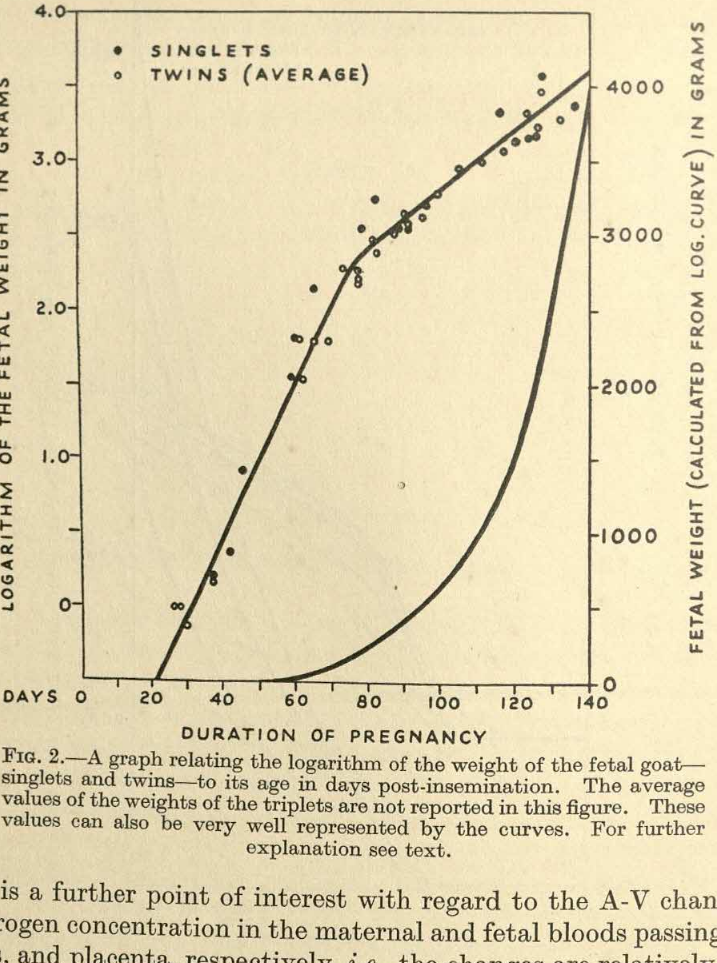

FIG. 2.—A graph relating the logarithm of the weight of the fetal goat—singlets and twins—to its age in days post-insemination. The average values of the weights of the triplets are not reported in this figure. These values can also be very well represented by the curves. For further explanation see text.

There is a further point of interest with regard to the A-V change in the amino nitrogen concentration in the maternal and fetal bloods passing through the uterus, and placenta, respectively, *i.e.*, the changes are relatively constant over the portion of the gestation period examined.

In this connection with the results presented in Table II, two regression lines expressed by the following equations were calculated:

$$D_m = 0{\cdot}00515A - 0{\cdot}130 \qquad (r = 0{\cdot}42,\ t_r = 1{\cdot}44,\ P_{t_r} = 0{\cdot}2 - 0{\cdot}1)$$

and

$$D_f = 0{\cdot}00765A - 0{\cdot}196 \qquad (r = 0{\cdot}25,\ t_r = 0{\cdot}83,\ P_{t_r} = 0{\cdot}5 - 0{\cdot}4)$$

where D_m and D_f are the A-V differences in mg./100 ml.—in the amino nitrogen concentration of the maternal and fetal bloods respectively, A the stage in pregnancy (in days), r the correlation coefficient with its Student's "t" and the probability value. The results indicate no significant correlation between the stage in gestation and the A-V difference in amino nitrogen in either the uterine or umbilical circulation. The differences appear to remain relatively constant in both circulations at least in the stages of development represented by the data in Table II. As the increased nitrogen requirements of the uterus and its contents do not appear to be met by the extraction of increased amounts of amino nitrogen from the maternal blood, the blood flow through the uterus must increase in proportion to the nitrogen requirements, *i.e.*, roughly in proportion to the curves representing the N requirements for the growth of the total tissues illustrated in fig. 3.

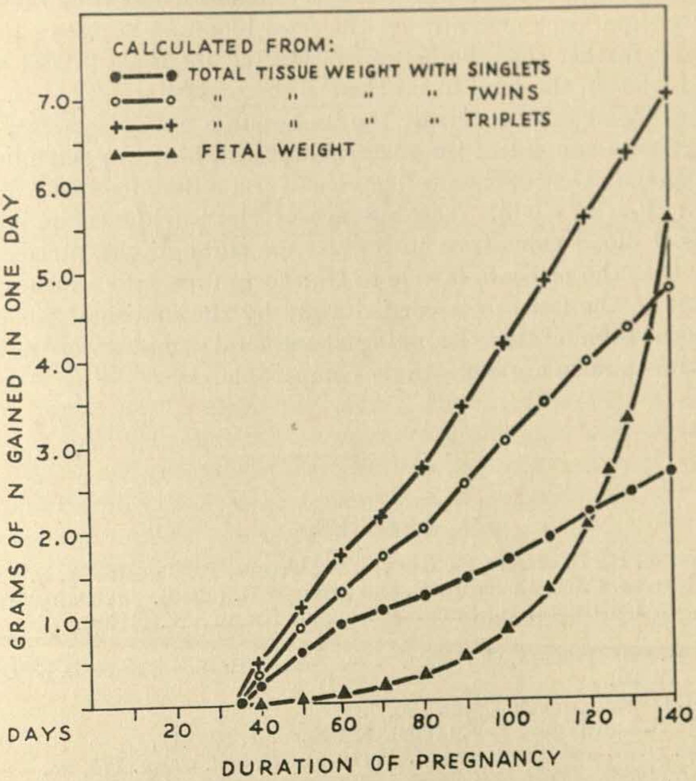

FIG. 3.—A graph relating the daily uptake of nitrogen in grams, by the uterus and its contents in pregnancies with singlets, twins and triplets respectively and by the fetus alone with the stage in gestation expressed in days following insemination. For further explanation see text.

Despite the relatively large amount of amino nitrogen removed from the maternal blood during pregnancy the level appears to remain relatively constant. Near the end of gestation in a goat of 40 kg. with a blood volume of 3 liters containing approximately 170 mg. of free amino nitrogen, with a uterine blood flow of 2000 ml./min. less than 30 min. is needed to remove all the amino acid from the maternal blood. On the contrary the level of the amino acids is relatively uniform in the different pregnant animals and quite similar to that characteristic of non-pregnant individuals. Further, the standard deviation of the concentration, expressed as a percentage of the mean, does not differ from the percentage standard deviation of the protein concentration and is about half that of the standard deviation of the values of urea. These considerations suggest that the amino acid level in the blood is regulated with a precision equal to that of the plasma proteins.

Finally the data presented in Table IV demonstrate that urea is lost in statistically significant amounts by the fetal blood as it passes through the placenta and further that the concentration (as De Bella [1958] had shown earlier) is higher in the fetal blood then in the maternal. That the urea lost by the fetal blood is taken up by the maternal is not so evident. (Though the average urea content of the maternal arterial blood appears in the table to be higher than that of the uterine venous the difference is not statistically significant: $P_t = 0{\cdot}7 - 0{\cdot}6$). The absence of clear evidence indicating that the maternal blood gains urea in its passage through the uterus raises the possibility that the placenta is able to transform urea into another substance that returns to the fetus or is carried away by the maternal blood. But it must be borne in mind that the maternal and fetal samples were not drawn at the same time and so are not strictly comparable.

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STUDIES ON THE OXYGEN UPTAKE AND EFFICIENCY OF CLIMBING OF TENSING NORGAY AND OTHER SUBJECTS.

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The oxygen uptake and efficiency of climbing of Tensing Norgay, the first man to climb Mount Everest, were compared with three other subjects during climbing hills with loads at an altitude of about 7000 ft. above sea-level. Each subject performed four experiments involving work of different grades of severity. The superiority of Tensing Norgay over other subjects was shown by the fact that his mechanical efficiency fell much less than that of the other subjects, as the climbing became more arduous.

MANY investigators have reported their observations on the respiratory responses and efficiency of walking uphill under natural outdoor conditions at altitudes well above the sea-level [Schumberg and Zuntz, 1896; Loewy, Loewy and Zuntz, 1897; Durig and Zuntz, 1904]. Douglas and co-workers [1913] made a detailed observation on the respiratory performances at Pike's Peak (14,100 ft.) using Douglas bags for the collection of expired air. In recent years Durnin [1955] used a new type of gas-meter (the Max-Planck respirometer) and studied the oxygen consumption, energy expenditure and efficiency of climbing of two subjects on Ben Lomond in Scotland.

The present work was undertaken to compare the efficiency of Tensing Norgay and of other subjects during climbing hills with loads under natural outdoor conditions at an altitude of about 7000 ft. (2·1 km.) above sea level.

METHODS

Subjects.—Four healthy subjects participated in the experiments, two of whom were from Calcutta and the other two (including Tensing Norgay) were natives of Darjeeling. The particulars of the subjects are given in Table I.

TABLE I.—SUBJECTS

Subject	Age (years)	Height (cm.)	Weight (kg.)	Remarks
A	38	168	69	Sedentary habits. Never been to the hills.
B	38	168	77	Athlete and champion swimmer.
C	28	153	50	Taxi-driver of Darjeeling. Sedentary habits.
D	48	175	63	Tensing Norgay, climbed Mt. Everest in 1953.

Site and Gradients.—The experiments were performed on two separate hills at Darjeeling with gradients of 1 in 8 (G1) and 1 in 6 (G2) respectively. The broad regular paths on these gradients were marked at measured intervals and with a little practice the subjects could cover a linear distance of 429 m. (1408 ft.) on either gradient in 8 min. The linear velocity of each subject was thus 3·22 km./hr. (2 m.p.h.) during any experiment. The rate of ascent on G1 was 6·71 m./min. and on G2 was 8·95 m./min.

Loads.—Empty cylinders were used as loads and were placed horizontally at the back by specially designed straps suspended from the shoulder. Two different loads were used in different experiments; viz. L1 = 7·3 kg. and L2 = 12·8 kg. (including the weight of the apparatus).

Apparatus.—A Max-Planck respirometer was used for measuring the pulmonary ventilation and for collection of an aliquot part of expired air for analysis. The apparatus is essentially a light-weight dry gas-meter with a device for collecting a suitable fraction of expired air in a rubber bag [Kofranyi and Michelis, 1940]. The apparatus was calibrated as recommended by Durnin [1955].

The micro-gas analyzer designed by Scholander [1942] was used for analysis of the expired air.

TABLE II.—PLAN OF THE EXPERIMENTS

Subjects	Days			
	1	2	3	4
A	L1G1	L1G2	L2G1	L2G2
B	L1G2	L2G1	L2G2	L1G1
C	L2G1	L2G2	L1G1	L1G2
D	L2G2	L1G1	L1G2	L2G1

Plan of the Experiments.—The two loads and the two gradients could be combined in four different ways, viz.: L1G1, L2G1, L1G2 and L2G2 respectively. It was thus possible to design four different experiments with each subject. The sixteen experiments on four subjects were arranged on four different days in the form of a 4 × 4 Latin square to facilitate statistical analysis. Table II shows the arrangement of the experiments.

The experiments were performed on 26th, 27th, 29th and 30th September 1957, in the morning after a light breakfast. The barometric pressure on these days varied between 592 mm. and 596 mm. of Hg. The ambient temperature varied between 14° C. and 15° C. and the relative humidity varied between 90 and 95 per cent. Each subject climbed steadily at a uniform speed of 3·22 km. per hour (2 m.p.h.) for 8 min. during each experiment. The volume of the expired air was measured and an aliquot sample was collected in the last 3 min. of the experiment when the subject was in the "steady state".

RESULTS

The data on ventilation, oxygen consumption, work done and mechanical efficiency of the different subjects arranged in order of severity of the work done are presented in Table III. The mechanical efficiency has been calculated from the caloric equivalent of external work divided by actual energy expenditure during work and expressed as a percentage. All gas volumes are expressed corrected to S.T.P.D.

DISCUSSION

The values of the oxygen consumption per kg. of body weight per minute during L2G2 experiments were 39·2, 28·2, 54·2 and 26·3 ml. respectively for the subjects A, B, C and D. The limits of oxygen consumption for an ordinary man as stated by Åstrand [1956] are about 41 ml./kg./min. for a man between

30 and 40 years of age; 53 ml./kg./min. for a man below 30 years of age; and 39 ml./kg./min. for a man between 40 and 50 years old. It will be seen, therefore, that the subjects A and C almost reached their limit of aerobic metabolism while climbing with the heavier load (L2) on the steeper gradient (G2). Subject B, and more particularly subject D (Tensing Norgay) had still in reserve some of their capacity for aerobic metabolism, under identical experimental conditions.

The mechanical efficiency of climbing of the subjects showed variations in different experiments. The subjects from Calcutta (A and B) climbed most efficiently on the lower gradient with lighter loads (L1G1 experiments). The mechanical efficiency of these subjects, however, deteriorated gradually and progressively as the climbing experiments became more and more strenuous. Subject C, a native of Darjeeling, contrary to expectation had, in general, a low mechanical efficiency in all the experiments which declined to as low a figure as 10 per cent in the most strenuous climbing experiment (L2G2). Tensing Norgay (Subject D), as expected, performed all the experiments with high mechanical efficiency. It is of interest to note that the superiority in the mechanical efficiency of hill-climbing of Tensing Norgay over the other subjects became more and more evident as the climbing conditions became more and more difficult (fig. 1).

TABLE III.—RESPIRATORY EXCHANGES, WORK DONE AND MECHANICAL EFFICIENCY DURING HILL-CLIMBING

Subject	Work schedule	Ventilation l./min.	Oxygen consumption ml./min.	Energy expenditure cal./min.	Work done kg.m./min.	Gross mechanical efficiency per cent
A	L1G1	20·4	1036	4·90	512	24
	L2G1	24·1	1250	6·16	549	21
	L1G2	42·1	1972	9·90	682	16
	L2G2	56·5	2700	13·50	732	13
B	L1G1	20·0	1078	5·30	566	25
	L2G1	24·2	1322	6·36	602	22
	L1G2	37·5	2005	9·93	753	18
	L2G2	45·5	2175	10·95	804	17
C	L1G1	20·3	940	4·70	385	19
	L2G1	22·1	1008	4·95	421	20
	L1G2	28·2	1418	7·00	513	17
	L2G2	49·6	2710	13·40	562	10
D	L1G1	17·9	985	4·75	472	23
	L2G1	18·9	1052	5·06	509	24
	L1G2	26·4	1468	7·10	629	21
	L2G2	29·9	1658	8·12	678	20

It might be of some significance that both the hill people climbed more efficiently on the lower gradient (G1) with heavier load (L2) whereas both the subjects from Calcutta could climb most efficiently only with the lighter load (L1) on the same gradient (G1).

The low mechanical efficiency of subject C (a native of Darjeeling) can perhaps be understood when it is remembered that by virtue of his profession as a taxi-driver, he was unused to climbing. The hill people habitually carry the loads on their back supported by a strap round the forehead. The loads in these experiments were suspended from the shoulder in a quite different way. Passmore and Durnin [1955] quote Bedale's observation to indicate that the method of carrying loads does influence the energy expenditure of the subject. It seems probable, therefore, that the sedentary nature of the occupation of subject C, coupled with the fact that he carried the loads in a way he was not accustomed to do might explain his low mechanical efficiency.

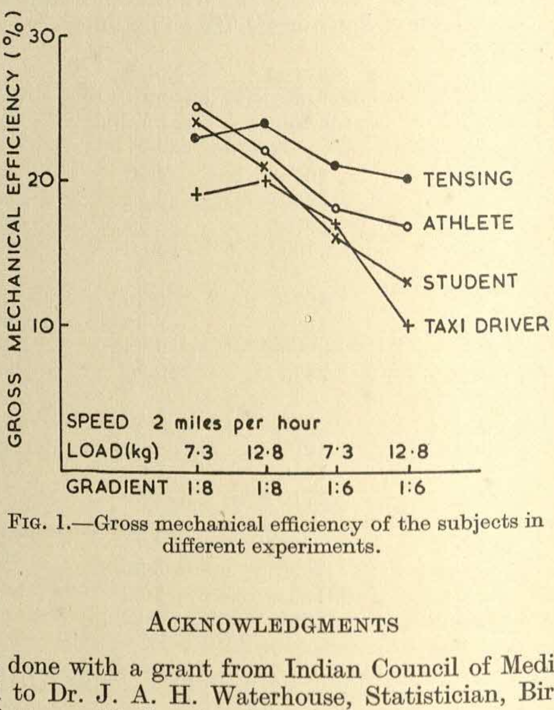

FIG. 1.—Gross mechanical efficiency of the subjects in different experiments.

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UPTAKE OF POTASSIUM AND SODIUM BY PARTS OF PACKED HUMAN BLOOD CELL COLUMN.

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The ^{24}Na, Na and K concentrations in portions of cell columns, packed from radio-active Krebs saline medium and mechanically separated from each other after centrifugation, decrease from the top downwards. The ^{42}K exchange of cells at the bottom is greater than that of cells in the middle, but less than that of cells in the top portion. These differences are not due to variations in the volume of medium trapped by the cells, to white cells, or to differences in red cell water content. Analysis of the tracer uptake curves suggests that there are at least two kinds of cell (which may be extremes of a continuum): one, relatively scarce and found progressively less frequently away from the top of the cell column having high K and Na concentrations and movement rates: the other, more numerous, with lower concentrations and movement rates. These differences may be related to the age of the cells: their importance is discussed.

It is convenient in quantitative studies to consider a population of red cells drawn from one individual as though it were homogeneous, but there has been evidence for some time that this simple view is not correct. It is believed, for example, that there are differences in the inorganic ion content of individual cells [Kruszyński, 1950], especially between those cells which are found at the top of the sedimented or centrifuged cell column and those at the bottom [Keitel, Berman, Jones and Maclachlan, 1955]. It has also been shown that enzyme activities [Pritchard, 1949; Jones and McCance, 1949; Allison and Burn, 1955] and ATP content [Rapoport, Guest and Wing, 1944] differ between young and old cells; and that more reticulocytes and young cells are found at the top of the column [Dreyfus, Schapira and Kruh, 1950; Chalfin, 1954; Borun, Figueroa and Perry, 1957]. Further, the consistency with which the kind of observations mentioned in the last group are made indicates that cells also differ in their sedimentation characteristics.

Recent analysis of tracer uptake by, and loss from, human blood cells [Solomon and Gold, 1955; Gold and Solomon, 1955; Joyce, Rayner and Weatherall, 1956; Solomon, Gill and Gold, 1957] has shown that exchange curves for potassium and sodium cannot be adequately described by equations for a first-order process. Hence the assumption that the cell potassium behaves homogeneously is invalid. Solomon and his colleagues have suggested that the inhomogeneity is intracellular [*i.e.*, that it is due to differences between parts of the individual cell], and have attempted to separate cell components having the appropriate characteristics [Solomon, Loucks, Pollen, Gill and Wolf, 1957]. On the other hand, the inhomogeneities might arise from differences between cells, as Creese, Neil and Stephenson [1956] have suggested is the case with tracer uptake by rat diaphragm.

Unlike the cells of "fixed" tissues such as muscle, blood cells can be separated from each other in a number of ways, and examined for differences of this kind. The results of observations upon the tracer uptake of cells separated by centrifugation are reported here. A preliminary account has already been given [Joyce, 1958].

METHODS

Blood was freshly drawn from the antecubital vein of healthy male human volunteers into a syringe containing solid heparin ("Pularin", Evans, 0·5 mg./ml. of blood drawn). The blood was centrifuged three times for 5 min. each at about 750 g, the supernatant being removed on the first two occasions and replaced by fresh Krebs saline solution (NaCl 0·120 M, $NaHCO_3$ 0·025 M, $CaCl_2$ 0·0025 M, $MgS0_4$ 0·0012 M, KCl 0·0042 M, dextrose 0·011 M). Isotonic solutions of ^{24}NaCl or ^{42}KCl were prepared from the spectroscopically pure carbonates irradiated at A.E.R.E., Harwell. These were substituted in the medium as required, for equivalent volumes of the inactive material.

Three methods of separating the cells were used.

Method 1.—After the third spinning, successive layers of cells, each containing about 5 ml. of cells, were removed by pipette to separate conical flasks. To these a sufficient volume of medium containing ^{42}KCl or ^{24}NaCl was later added to give an approximately 20 per cent (v/v) suspension of cells. Samples of 1 ml. were taken at intervals after the start of incubation at 37° C., and were treated as described below for the unseparated cell samples of methods 2 and 3.

Methods 2 and 3.—After the third spinning, the cells were resuspended in fresh inactive medium, and portions of this suspension were transferred to conical flasks for incubation at 37° C. with Krebs saline containing ^{42}KCl or ^{24}NaCl. At intervals after the beginning of incubation, 1 ml. portions were removed for centrifugation at 3000 rev./min. for 25 min. in modified centrifuge tubes [Joyce, Moore and Weatherall, 1954] with capillary portions of approximately 2 mm. internal bore and 50 mm. in length. The exact volumes contained in measured lengths of the capillary portions were determined by calibration with mercury or distilled water. During spinning, the bottom of the tube was at a radius of 150 mm. from the centre of the centrifuge head. The supernatant was removed by suction, and the interface was washed with 5 per cent (w/v) dextrose solution. In method 2, three successive and approximately equal portions of the packed cell column were removed by Pasteur pipette to separate volumetric flasks: the part of the tube from which each portion had been taken, and the pipette, were thoroughly washed with dextrose solution, and the washings were added to the appropriate flask. In method 3, the capillary of the tube was divided into three approximately equal parts. These were separated by breaking cleanly at the required points, and the contents of each section were washed with 0·1 per cent (v/v) 0·880 NH_4OH solution into separate volumetric flasks.

In experiments using methods 2 and 3, some samples were transferred to flasks without being divided, as a check on recoveries. In experiments using all three methods, samples of the whole suspension (containing cells and medium) were also taken. All solutions were diluted to either 25 or 50 ml. with 0·1 per cent (v/v) 0·880 NH_4OH solution.

The radioactivity of solutions prepared in this way was estimated in duplicate with liquid counters, using conventional methods. Potassium and sodium were estimated in duplicate with an "Eel" flame photometer (Evans Electroselenium, Ltd.) and oxyhæmoglobin concentrations relative to a standard calibration curve with the Hilger & Watts "Spekker" photometer and Chance OG 1 filters (maximum transmission at 5300 A). The absolute amount of hæmoglobin contained in the solutions was not determined. Red and white cell counts were made in the conventional ways [Wintrobe, 1956]. In most experiments white cells were largely removed by suction of the buffy coat during washing or after centrifugation of the samples. The volume of the medium trapped by the packed cell column was determined with inulin: cell samples were centrifuged from a medium that contained 2·5 per cent (w/v) inulin in addition to its usual constituents. After removal of the supernatant, portions of which were retained as standards, the cells were resuspended in inulin-free medium and again centrifuged. The concentration of inulin in the new supernatant was compared with that in the original by the colorimetric method for fructose of Bacon and Bell [1948]. The reagents used hydrolyzed the inulin satisfactorily, and the method was used without modification. Ilford 602 filters (maximum transmission at 4700 A) were used in the "Spekker" photometer.

RESULTS

The numbers of white cells did not differ systematically or significantly between the portions of separated cells (Table I). They were reduced to about 6 per cent of the number present in the original whole blood: more vigorous treatment, or further washing, would have caused loss of red cells which it was particularly desirable to keep, as well as loss of cell potassium [Davson and Danielli, 1938]. There were no significant differences in the water content or in the colour indices of the three portions in any experiment. The effects of small differences in the volumes of the portions, as sampled, were therefore eliminated with the aid of the relative hæmoglobin concentration. This was more accurately estimated than the packed cell volume, of which it was an indirect measure, and the values given subequently thus represent the contents of standard volumes of packed cells. The propriety of this adjustment is discussed later.

The volume of medium trapped by packed cells was rectilinearly related to the volume of the cells over the range of column lengths that was used (fig. 1).

The volume of supernatant trapped amounted to 4·65 ±0·09 per cent (s.e. of seventeen determinations), and the appropriate deduction was therefore made from the adjusted values of the cell potassium, sodium, ^{42}K and ^{24}Na. The total recovery of material separated into portions was not significantly less than the material contained in undivided cell columns (p > 0·1: range 94–107 per cent of undivided totals).

TABLE I.—RED AND WHITE CELL COUNTS, WATER CONTENT AND RELATIVE HÆMOGLOBIN CONCENTRATION OF PACKED CELL PORTIONS

Mean Values ± s.e. (no. of estimates).

Portion	Total solids per cent wet wt.	Hb concn. arb. units mm.$^{-3}$ cells	Red cells 10^{-6} mm.$^{-3}$ cells	White cells 10^{-3} mm.$^{-3}$ cells
Top . .	36·0 ±0·85(2)	24·9 ±0·99(8)	9·48 ±0·37(10)	0·78 ±0·14(3)
Middle . .	39·1 ±1·56(2)	25·5 ±0·72(8)	10·73 ±0·23(10)	1·12 ±0·13(3)
Bottom .	36·5 ±0·57(2)	26·9 ±0·50(8)	9·89 ±0·22(10)	1·13 ±0·17(3)
Original blood	..	..	10·34 ±0·23(10)	15·36 ±0·22(6)

Probability of chance difference between top and bottom portions is > 0·05 in all cases.

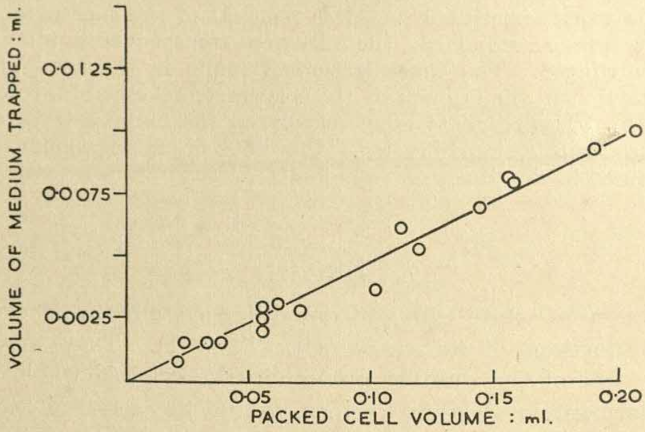

FIG. 1.—Volume of medium trapped by cell columns packed at mean centrifugal force of 1500 *g*. Regression line fitted by method of least squares: $y = 0{\cdot}0465x(\pm 0{\cdot}0009$: s.e. of 17 determinations).

Fig. 2 and Table II show the sodium and potassium concentrations of cells packed in the top, middle and bottom thirds of the column.

Fig. 3 shows the corresponding uptake of ^{24}Na and ^{42}K, observed on separate occasions. The differences between the ^{24}Na concentrations are in the same direction as those in total sodium.

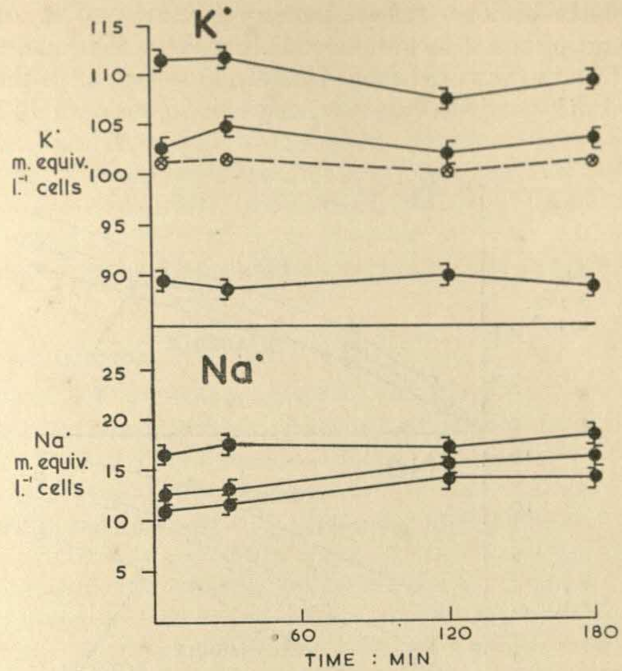

Fig. 2.—K and Na concentrations of top, middle and bottom thirds of packed cell columns. Mean and s.e. of duplicate determinations. ⊗ - - - ⊗: K concentration of unseparated cells: s.e. of triplicate determinations smaller than symbols used for mean. Na concentration of unseparated cells omitted (not distinguishable from that of middle portion).

Table II.—Sodium and Potassium Concentrations in Packed Cell Portions

All values: mean m.equiv/l. cells ±s.e. (no. of estimates).

	Experiment No.					
Portion	11.12		25.3		25.4	
	Na	K	Na	K	Na	K
Top .	32·3 ±2·49(6)	108·3 ±1·78(6)	17·9 ±0·71(4)	116·5 ±1·20(4)	16·6 ±0·23(3)	104·5 ±1·01(6)
Middle .	26·3 ±1·20(6)	96·8 ±1·71(6)	14·0 ±1·20(4)	108·3 ±0·69(4)	15·5 ±0·84(3)	99·5 ±0·71(6)
Bottom .	25·0 ±2·27(6)	84·0 ±0·97(5)	13·2 ±0·87(4)	93·9 ±0·42(4)	12·2 ±0·39(3)	87·6 ±1·04(6)
Mean .	27·9	96·4	15·0	106·2	14·8	97·2

Probability of chance difference between all portions in any column: < 0·001 for each experiment.

Probability of chance difference (top-middle) for K: < 0·01 > 0·001.

Probability of chance difference (top-middle) for Na: < 0·15 > 0·02.

Probability of chance difference (middle-bottom) for K: < 0·001.

Probability of chance difference (middle-bottom) for Na: < 0·35 > 0·01.

The differences between the contents of ^{42}K are not so simply related. The bottom portion usually contained slightly more ^{42}K than the middle at all times, and had always exchanged more potassium than had the middle (Table III). Both portions, however, contained considerably less ^{42}K than did the top.

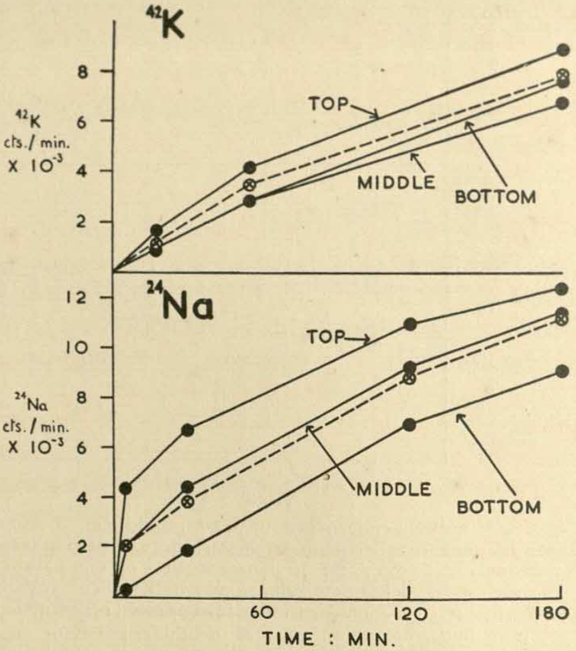

FIG. 3.—^{42}K and ^{24}Na content of top, middle and bottom thirds of packed cell columns. ⊗ - - - ⊗: unseparated cells. At 180 min., ^{42}K content of bottom portion was higher than that of middle

TABLE III.—^{42}K EXCHANGE BY BOTTOM AND MIDDLE PORTIONS

Expt.	Time min.	Per cent middle exchanged	Per cent bottom exchanged	$\frac{\text{Middle}}{\text{Bottom}}$
11.12	20	2·57	2·90	0·886
		2·87	3·30	0·870
		2·47	3·23	0·765
	55	8·64	9·75	0·886
		9·40	11·44	0·822
		9·07	9·69	0·936
	200	23·44	26·90	0·871
		22·17	32·46	0·683
25.1	180	13·74	15·46	0·889
		14·33	15·63	0·917
25.4	100	9·54	10·01	0·953
		9·31	9·96	0·935
	150	14·00	14·48	0·967

Mean: 0·875
±0·0212 (s.e.)
$p < 0{\cdot}0001$

Figs. 2 and 3 are based on experiments using methods 2 and 3. The smallest differences between portions were observed in an experiment which employed method 1, where the effective separation of the cells was carried out at a much lower centrifugal force (*c.* 750 g), and in conventional centrifuge tubes of much wider bore than those used in the other methods. Even here, however, the cell potassium concentration of the top and bottom portions differed by 3·4 ± 1·44 m.equiv/l. cells (mean and s.e. of four determinations). The ^{42}K concentrations differed by comparable amounts, and the differences were in the same direction as those found with the other methods of separation.

DISCUSSION

The observed differences in sodium and potassium concentration of portions of washed human blood cells are of similar magnitude to those reported by Keitel *et al.* [1955] for whole blood. The present work shows that these differences are reliable. (See Table II, which gives the probabilities that such results could have occurred by chance.) Differences in the ^{42}K or ^{24}Na concentration of the top and remaining portions are large, consistent at all times and significant at $p < 0{\cdot}001$ for individual experiments. The same is true of the differences between the ^{24}Na concentrations of the middle and bottom portions. However, the differences in the ^{42}K concentrations of the middle and bottom portions are usually small, not significant at $p < 0{\cdot}1$, and almost always in the reverse direction from that which would have been expected from the gradient for potassium. When the observations made during the first 3 hr. of incubation in four experiments are combined, the mean exchange of potassium by the middle portion is found to be only 87·5 ± 2·12 per cent (s.e. of thirteen determinations) of the exchange by the bottom portion. This is significantly less than 100 per cent ($p < 0{\cdot}0001$) (Table III).

The hæmoglobin concentration differences of the portions were not observed to differ significantly in any experiment (Table I); but it is necessary to the argument, in view of the importance of these estimates, to consider the magnitude of the error likely to be introduced if there were in fact a hæmoglobin concentration gradient from the top downwards. The largest difference of the hæmoglobin concentration of any portion from the mean value for the undivided cells was never more than ± 6 per cent. If the values in Table II (means and variances) are adjusted accordingly, the differences between the potassium and sodium concentrations of the top and bottom portions are still significantly large ($p < 0{\cdot}05$ for sodium, $< 0{\cdot}01$ for potassium). Such an adjustment would of course also increase still further the disparity between the ^{42}K concentrations of the middle and bottom portions.

It is therefore clear that the cell samples under observation contain at least two components, and that these components cannot be identified with different parts of individual cells. By using washed cells, the present work has excluded the possibility that the inhomogeneity arises from differences in content of material which originates in the plasma and is associated with the cells on centrifugation. The count of the white cells which remained after removal of the majority did not vary in the same direction as the potassium and sodium content. It is shown below that they were also too few in number to account for the observations.

If the system contains two kinds of cell, observations on tracer uptake at different times allow the determination of the relative sizes of these components by graphical methods [Gellhorn, Merrell and Rankin, 1944; Ponder, 1951]. The relationship between time and the function $\ln(1 - S_t/S_\infty$ (where S_t = cell specific activity at time t, and S_∞ = cell specific activity when the distribution of tracer is uniform throughout the system) gives a curve which can be described with the aid of two exponential constants. The slope of the latter portion of the curve gives the rate-constant for movement of material between the medium and the larger of the two intracellular compartments. When this line is retrapolated to the ordinate, the exponent of the intercept divides the ordinate in the proportion of the two intracellular components. Extraction from the first part of the curve (replotted after deduction of the extrapolated values of the latter part) of the rate-constant for movement between the medium and the small intracellular component is unreliable in the situation under consideration, because exchange between these phases is very fast, and timing of the separation of the cells from the medium cannot be estimated with the necessary precision.

TABLE IV.—COMPONENT SIZES AND K, NA MOVEMENT IN PORTIONS

Portion	Potassium			Sodium		
	Small component		Slow movement	Small component		Slow movement
	per cent total	m.equiv/l. cells	m.equiv/l. cells/hr.	per cent total	m.equiv/l. cells	m.equiv/l. cells/hr.
Top . .	1·1	1·2	1·67	39·3	7·0	3·5
Middle .	0·9	0·9	1·58	9·8	1·4	5·1
Bottom .	0·4	0·3	2·04	0·01	0·0	4·7
Unseparated Cells .	0·9	0·9	1·97	13·5	2·0	5·0

Estimates of the component sizes and of the slow movement (between medium and large component) are shown in Table IV. The slow movements do not differ systematically between portions. About two and a half times as much sodium as potassium is moved in unit time. The sizes of the small components, whether as absolute quantities or as proportions of the total, differ systematically between portions. They are not of the same size for potassium as for sodium, although they vary in the same directions.

The total potassium content of the top portion is greater by 16·9–24·3 m.equiv/l. cells (Table II), and the corresponding difference in the sizes of the calculated fast component is 0·9 m.equiv/l. cells (Table IV). Differences in the size of this component therefore do not account for the greater part of the difference in total potassium content. As the white cell potassium content of whole blood is less than 1·5 per cent of the total [Solomon and Gold, 1955], the small component is much too large to represent the white cell potassium content of experimental suspensions in which the white cell count was reduced to about 6 per cent of the number present in the original whole blood (Table I). If the potassium content of reticulocytes resembles that of mature red cells, the fast component might consist of reticulocytes. The number of these cells decreases from about 6 per cent of the total at the top of the cell column to zero at the bottom [Keitel *et al.*, 1955]. On the other hand, the uptake of more ^{42}K by the bottom than by the middle portion (Table III) is evidence against this simple explanation.

In the case of sodium, the difference between the contents of the top and bottom portions lies between 4·4 and 7·3 m.equiv/l. cells (Table II): Table IV shows a difference of 7 m.equiv/l. cells. There is thus good agreement; but the size of the fast component is nevertheless too large to represent reticulocytes (if the sodium content of the latter resembles that of mature cells); nor can it be correlated with the variation in the number of white cells in each layer.

It must be borne in mind that the "parallel" type of arrangement discussed above is not unique. Other solutions of the equations relating tracer movements to time can be obtained, with the aid of additional assumptions, for the "series", "series-parallel" and other more complex types of arrangement [Robertson, 1957]. But the observations described in the present work could not arise from systems in which the inhomogeneity is purely intracellular [Solomon and Gold, 1955], or is restricted to differences between the pathways by which ions move between homogeneous cells and uniform environment [Shaw, 1955; Glynn, 1956, 1957]. They do not exclude the possibility that such additional complexities also exist. Intracellular differences have recently been sought, but have not yet been demonstrated by direct methods [Solomon *et al.*, 1957].

Table III shows that the combined content of sodium and potassium decreases from the top portion downwards. Keitel *et al.* [1955] report that the contents of chloride and phosphate ions vary in the same direction as do those of sodium and potassium. It is therefore possible that the lower cells are hypotonic with respect to those above them, if not to the medium. The greater resistance to osmotic lysis shown by older cells [Cruz, Hahn, Bale and Balfour, 1941; Simon and Topper, 1957] may be due to a smaller content of osmotically active material, since young cells are believed to occupy a progressively smaller proportion of the packed cell column from the top downwards (Dreyfus *et al.*, 1950; Borun *et al.*, 1957].

A relationship between cell age and differences in composition therefore seems very probable, and suggests that simultaneous study of the organic and inorganic metabolism of separated cell portions may give useful information about the way in which red cells move sodium and potassium. The kind of experiment described above involves less disturbance to the cell than do certain other more frequently practised techniques.

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A CHEMICAL ANALYSIS OF THE SEMINAL AND BLOOD PLASMAS OF THE COCK.

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The concentrations of sodium, potassium and calcium have been determined in seminal and blood plasmas of the cock. A few estimations of magnesium, copper and zinc are also reported. The concentration of potassium in seminal plasma is more than twice that of blood plasma, calcium is lower and sodium not significantly different. The potassium concentration in seminal plasma probably changes with age; this finding is discussed. Citric acid is absent from the seminal plasma and the chloride concentration is 59 m.equiv/l. The absence of citric acid in cock semen, and the totally different concentrations in seminal plasma of some inorganic ions between domestic mammals and the cock could be accounted for by the lack of accessory reproductive organs in the latter. The ionic composition of previously suggested, but inefficient, semen storage diluents is contrasted with the findings here recorded in relation to seminal plasma. A comment is made on the electroneutrality of the latter.

Fowl semen has proved particularly difficult to store *in vitro* for artificial insemination purposes. The diluents used, were designed by arbitrary modification of mammalian semen diluents and physiological salines [Bogdonoff and Shaffner, 1954; Takeda, 1954; Schindler, Weinstein, Moses and Gabriel, 1955]. Such diluents have maintained spermatozoa reasonably fertile for only a few hours. It is possible that the ionic compositions of these diluents create an environment unfavourable to the spermatozoa.

Hitherto no attempt has been made to find out whether fowl seminal plasma shows peculiar features of chemical composition, or how far a diluent must correspond to the natural fluid in order to allow the spermatozoa to survive for prolonged periods. This present paper describes an exploratory examination of some electrolytes of seminal plasma.

Materials and Methods

Seminal Plasma.—Clinically healthy and reproductively active Brown Leghorn cocks were housed in galvanized battery cages and received the Poultry Research Centre diet [Bolton, 1958]. The method of collecting semen directly from the ejaculatory ducts has been described by Lake [1957 *a*]. For an analysis of sodium, potassium and calcium a range of semen samples was collected into specially cleaned pyrex tubes. Composite samples were collected on two occasions from a group of thirty cockerels aged 7 months, and once from a group of 13 months old cocks. Individual samples were collected from nine cocks of various ages; four 7 months, one 15 months, two 18 months and two 22 months old. Semen from some of the latter was also required for another investigation of organic constituents; since a single ejaculation (0·25–0·35 ml.) was insufficient for all the analyses, samples were collected periodically and the plasma separated, bulked and stored at −25° C. Each sample was centrifuged at 1500 g. for 20 min. at 5° C. within 5–10 min. after collection and the plasma immediately pipetted into cleaned pyrex storage tubes before the spermatozoa began to diffuse upwards. Care was taken to prevent evaporation and contamination of the sample during cold storage.

Pooled residues from the above samples were used for determination of magnesium, copper and zinc.

Since citric acid and chloride are two substances which form the bulk of anionic material in the seminal plasma of mammals, including man, examinations were made for their contribution in the cock. Chloride was determined in seminal plasma from composite and individual samples collected separately from the 7 months old cockerels mentioned above, and a search was made for citric acid in fresh samples.

Blood Plasma.—Blood was taken from some of the 7 months old cockerels, the 15 months, a 23 months and a 29 months old cock for analyses of the plasma for sodium, potassium and calcium. Magnesium, copper and zinc determinations were made on pooled residues. Blood samples were taken from the wing vein into pyrex syringes moistened with heparin solution; the plasma was separated and stored under the same conditions as for seminal fluid. No special precautions were used to prevent loss of carbon dioxide beyond speed of working.

Analytical Methods

1. Sodium, potassium and calcium were determined in the same sample of plasma (volume 0·4 – 1 ml.). Doubly (glass) distilled water was added to bring the volume to 5 ml. and the calcium, precipitated by addition of 1 ml. of saturated ammonium oxalate solution, was determined by a modification of Powell's [1953] method. Sodium and potassium were determined in aliquots of the supernatant fluid by direct flame photometry after they had been diluted to give suitable concentrations (1·5–3·0 mgm. Na/Litre and 2·0–6·0 mgm. K/L) using an EEL flame photometer. Blank values were negligible.

2. Magnesium was determined by the modified [Hawk, 1947] method of Dennis [1922] and copper and zinc by dithizone procedures [Butler, 1952; Butler and Newman, 1956].

3. Chloride was determined in 0·4 ml. aliquots of seminal plasma by the method based on that of van Slyke [1923] using N/50 thiocyanate and silver nitrate solutions. The test for citric acid was made using the method described by Mann [1953]. 0·1 ml. samples of seminal plasma from individual cocks were deproteinized by adding 1·4 ml. distilled water and 1·5 ml. 20 per cent trichloroacetic acid.

RESULTS AND DISCUSSION

No citric acid was detected in seminal plasma. The average chloride concentration was 59 m.equiv/l. (range 56·3–60·6). From Table I the potassium values for the older cocks is significantly higher ($p < 0{\cdot}001$) than for the younger ones while the sodium and calcium values show no significant differences. On present evidence this difference does not necessarily indicate a change with age since the samples were collected from the two groups during different periods of the breeding season, viz., after the peak and at the beginning respectively. However, it is known that the fertility and hatchability of eggs decline towards the end of the breeding season [Blyth, 1945] and with advancing years [Landauer, 1951]. While a reduction in semen volume and in the activity of the oviduct and ovary may be contributory factors in both cases, there may also be a gradual adverse physiological change in the semen. Changes in the spermatozoa themselves might be reflected in changes in the ionic composition of the plasma.

Our results indicate that the diluents previously used for fowl semen contained unphysiological amounts of some inorganic ions, particularly when based on mammalian physiological salines and semen diluents. For instance, Tyrode, Ringer and Ringer-Locke solutions contain a lower concentration of potassium (2·5–5·3 m.equiv/l.) than seminal plasma and a much higher chloride content (147–155 m.equiv/l.). The calcium level of the saline solutions tends to be more physiological in amount, but the magnesium level of Tyrode solution (2 m.equiv/l.) is lower than that of seminal plasma.

Table I.—Concentrations (Means ±S.D.) of Sodium, Potassium and Calcium, in m.equiv/l., in the Seminal and Blood Plasmas of the Cock

	No. of samples	Age of birds (months)	Calcium	Sodium	Potassium
Seminal plasma	7	7–13	3·3±0·45	171±8	12·6±1·1
			(2·7–3·8)	(161–182)	(10·7–14·3)
	5	15–22	3·8±0·85	187±16	17±1·7
			(2·9–5·0)	(167–208)	(15·6–20)
Blood plasma	5	7	5·3±0·15	171±4	4·9±0·4
			(5·1–5·5)	(165–177)	(4·6–5·6)
	3	15–29	5·5±0·1	168±8	4·7±0·4
			(5·4–5·6)	(163–177)	(4·4–5·0)

A comparison of our results with the published values for mammalian seminal plasma [Rothschild and Barnes, 1954; Mann, 1954] is of little value owing to the wide variation in the latter. This is probably due to the absence of, or differences in the degree of development of, the accessory reproductive glands in the various species. The electrolytes present in the ejaculated semen of mammals are mainly contributed by these glands; since these are absent in the cock differences in the composition of the seminal plasma are to be expected. For instance, the absence of citric acid in fowl seminal plasma could be due to the lack of a prostate gland [Lake, 1957 *b*]. The values for zinc reported for ram and human plasmas [Mann, 1945; Mawson and Fischer, 1953] greatly exceed that of the fowl (·057 m.equiv/l.); zinc is contributed mainly by the prostate gland.

The values for sodium potassium and calcium in cock blood plasma are given in Table I, and are in general agreement with previous findings [Benjamin and Hess, 1933; Dyer and Roe, 1934; Morgan and Chichester, 1935; Maizels, 1954] bearing in mind that there may be breed and sex differences in the content of some of these constituents in the blood. A comparison of the results for seminal and blood plasma reveals important differences. The potassium content of the seminal plasma is more than twice that of the blood plasma, as in the case of mammals. The magnesium level is higher, 7 m.equiv. compared with 2 m.equiv/l. but the calcium level is lower ($p < 0{\cdot}001$). There is no significant difference between the two sodium levels. The few results for copper and zinc do not indicate wide differences; the copper content of blood plasma and seminal plasma was 9·8 and 10 μ equiv/l. respectively, and zinc was ·048 and ·057 m.equiv/l. respectively. It should be noted that the needle used for the collection of blood had a brass hub and this may have caused some elevation of the true values of blood plasma copper and zinc [Butler and Newman, 1956].

In conclusion it is worth commenting on the electroneutrality of fowl seminal plasma. Lake and MacIndoe (unpublished observations) have shown that there is a high concentration of free glutamate in the fluid, and some of the same cocks were used in their work and ours; Table II shows the average concentration, from an analysis of eight samples, of the main electrolytes of the seminal plasma, the figure for glutamate being taken from Lake and MacIndoe. Citric acid is absent and the chloride level alone is insufficient to balance the cations, and so glutamate is likely to play an important part as anionic material. Further work is contemplated on the chemical composition of cock semen with a view to determining the metabolic processes underlying the activity of the spermatozoa, and it is hoped that such an investigation will ultimately lead to the creation of the best physical and chemical environment for the prolonged maintenance of fowl spermatozoa *in vitro*. Already the results of the present work have been used to compose a test diluent for semen, and very promising fertility results have been obtained after the insemination of semen which has been diluted and stored for 24 hr. at 2° C.

TABLE II.—ANALYSES, SEMINAL PLASMA OF COCKS

	Average concentration (mg./100 ml.) (1)	S.D. of (1) (2)	Range of (1) (3)	Average concentration (m.equiv/l.) (4)
Sodium .	393	17	379–428	171
Potassium	43	4	39–49	11
Calcium .	8	0·9	6·9–9·3	4
Chloride .	205	7	197–212	59
Glutamate	1033	91	890–1140	70

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ON THE HEART RATE DURING EXERCISE, THE ŒSOPHAGEAL TEMPERATURE AND THE OXYGEN DEBT.

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Heart rate, alveolar CO_2 tension and œsophageal temperature were measured in a single subject during exercise at eight different rates of work. An estimate of oxygen debt was made after 10 min. exercise at each rate.

The mean acceleration of the heart after the first 5 min. of exercise and the rise in œsophageal temperature due to exercise both increased as the rate of work progressed up to a steady state oxygen uptake of 2000 ml./min. Above this rate of work neither showed a further increase. A rising deep temperature may therefore be responsible for the slowly rising heart rate noted after 5 min. in moderate and severe exercise. It is presumably operative during the first 5 min. also, although its effect is obscured by the numerous other changes which take place during the early phases of adaptation. The heart rate during the first 5 min., after correction for the presumed effects of increasing temperature, was found to reach its steady state level within the first half-minute in the milder exercises. In more severe exercise this was not the case and the total deficit of heart beats during the first 5 min. was found to increase sharply above an oxygen uptake of 2000 ml./min. The oxygen debt rose steeply and the alveolar CO_2 tension fell as the rate of work increased above this level. The implications of these findings are discussed.

How the heart rate increases during exercise is still not precisely understood. The more obvious possibilities have recently been discussed by Harris and Thomson [1958] who presented a comparison between the heart rate responses to exercise of a group of healthy men aged 70–79 and a group of healthy undergraduates. It was suggested that an increasing heart rate between the end of the first half-minute and the end of the fourth minute of exercise might be determined by chemical factors, such as the products of partially anærobic muscular activity, or by an increasing venous return.

The object of the present study was to obtain more exact information about the possible relation between heart rate and chemical factors. It proved necessary to take account also of changes in body temperature.

METHODS

One of us (B. B. P.) was the subject for all the experiments. He is aged 24, fit though not in training, is 173 cm. tall and weighs 66 kg. when wearing canvas shoes, shorts and vest in which the experiments were done. He walked on a motor-driven treadmill at eight different rates of work: 3 m.p.h. and 4 m.p.h. on the level and 4 m.p.h. at gradients of 2·5, 5·0, 7·5, 10·0, 12·5 and 15·0 per cent. Preliminary experiments showed that these rates corresponded to oxygen uptakes of 950, 1300, 1520, 1800, 2080, 2360, 2650 and 2920 ml./min. during the steady state (fifth and sixth minutes) of exercise. Each exercise lasted 10 min. from a standing start, measured by an electric clock. After each exercise the subject rested for at least 20 min. before starting the next exercise. All experiments were done in the afternoon following a light lunch. In any one afternoon only one exercise at an O_2 uptake greater than 1800 ml./min. was done, and this last of all. This precaution made it reasonably certain that no exercise was begun before the oxygen debt from the previous exercise was fully repaid. Two electric fans were directed on the subject during each exercise, and room temperature was kept approximately constant at about 65° F.

Heart rate was measured with a cardiotachometer [Austin and Harris, 1957]. Alveolar air was collected during the sixth and seventh minutes of exercise with an end-tidal sampler [Harris, 1957] modified from that of Clark-Kennedy and Owen [1927]. Oxygen debt was assessed from the total oxygen consumption during the first 20 min. recovery, the subject sitting quietly in a comfortable chair. Not infrequently he fell asleep during this period. Expired air for the calculation of debt was collected in a 500 l. Douglas bag and measured with a wet gas meter. Alveolar and expired air were analyzed by the Haldane method. Duplicates were required to check to within 0·03 vol. per cent for both O_2 and CO_2.

Temperature measurements were made with a copper-constantan thermocouple in the œsophagus immediately behind the left atrium, the position being confirmed radiologically. Whether this method gave a true measure of left atrial temperature is uncertain. Hyperventilation at rest had no effect on the recorded temperature though a reduction might have been expected if the blood were appreciably cooled in passing through the lungs. Swallowing, on the other hand, caused an abrupt though variable fall in temperature. The subject therefore did not swallow for at least 20 sec. before each temperature reading was taken; the reading always became stable within this period.

RESULTS

Fig. 1 shows consecutive half-minute heart rates at each of the working intensities; each line is the mean of four experiments. The figure thus summarizes the results of thirty-two exercise periods. A slow rise in heart rate during the last 5 min. of exercise appears at intensities corresponding to O_2 uptakes of 1520 ml./min. and above. This rise [the *Ermüdungsansteig* or "fatigue rise" of Müller, 1950] was estimated for each exercise intensity as the slope of the regression line of heart rate on time during the last 5 min. of exercise and plotted against exercise intensity in fig. 3. The temperature

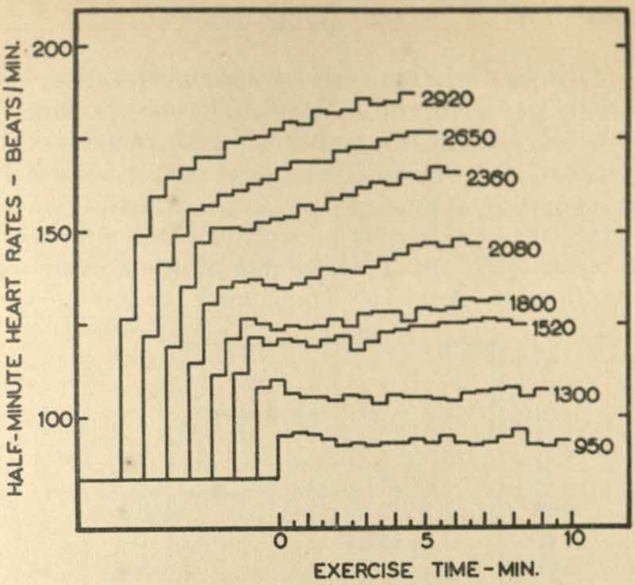

FIG. 1.—Mean consecutive half-minute heart rates, in beats per minute, at eight different exercise intensities. Mean values of four exercises at each intensity.

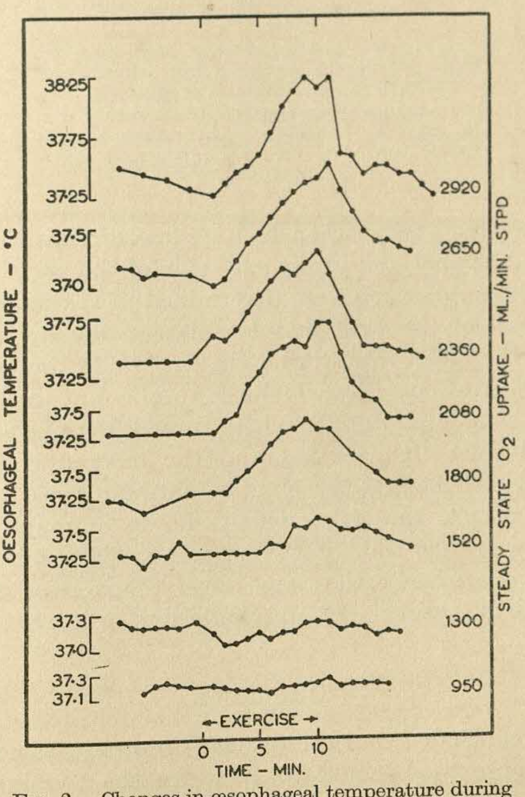

FIG. 2.—Changes in œsophageal temperature during exercise at eight different intensities.

changes are shown in fig. 2 and the total rise in temperature at each intensity, plotted against the rate of work, in fig. 3. The mean cardiac acceleration during the last 5 min. and the temperature rise due to exercise both increase up to an O_2 uptake of 2000 ml./min. and show no appreciable further increase between 2000 and 3000 ml./min.

The heart rate curves were next corrected for the *Ermüdungsansteig* by subtracting from successive mean half-minute counts a cumulative quantity determined from the slope of the last 5 min., after the method used by Müller

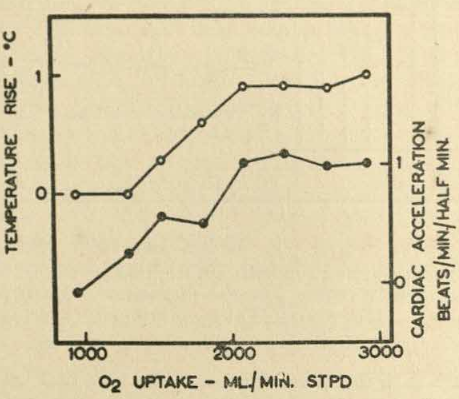

FIG. 3. ●——● Increase of heart rate, in beats per minute per half-minute, during the sixth to tenth minutes of exercise. ○——○ Rise of œsophageal temperature due to exercise. Abscissa: O_2 uptake during steady state of exercise (fifth and sixth minutes).

[1950]. Each corrected curve then had a slope of zero in its last half and the first half could be analyzed to the exclusion of the effect of *Ermüdungsansteig* upon it. Three functions were determined: (1) the absolute heart rate reached at the end of the fifth minute (plateau level); (2) the difference between (1) and the mean rate during the first half-minute; (3) the "pulse deficit", the number of beats by which the total count in the first 5 min. fell short of that in the second 5 min. These three functions are shown in fig. 4 together with alveolar CO_2 tension and the oxygen consumption during 20 min. of recovery. The first two show a continuous rise as the rate of work increases. In contrast, the pulse deficit shows no major increase until a working intensity of 2000 ml. of O_2 per minute is reached and then rises sharply. At approximately the same level of exercise the alveolar P_{CO_2} begins to fall and the recovery O_2 uptake begins to increase more rapidly.

DISCUSSION

Müller (1950] concluded that the *Ermüdungsansteig* was related to physical fitness in that more work could be done by the "trained" than by the "untrained" subject for the same "fatigue-rise". This conclusion was based on comparatively few observations of his own and of Christensen [1931]. Müller suggested no explanation for the "fatigue-rise" beyond stating that it appears, as a concomitant of fatigue, in exercises which are beyond the capacity of an individual to continue indefinitely.

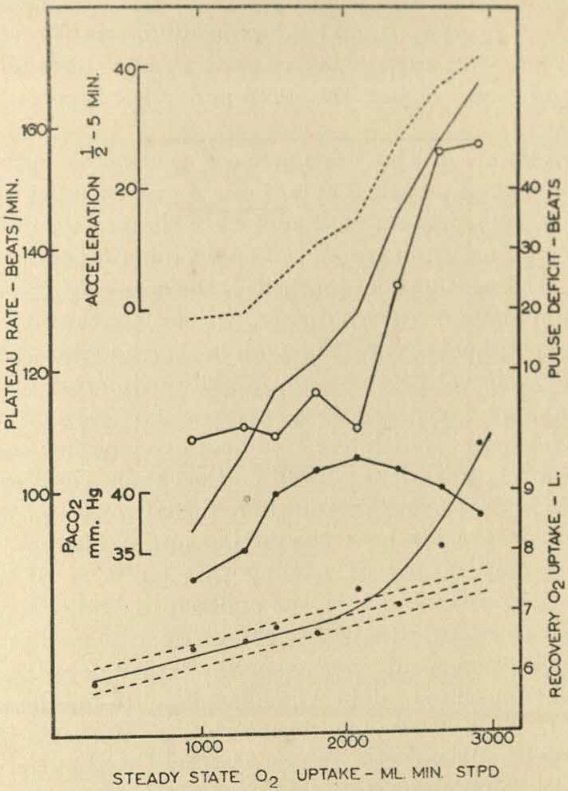

FIG. 4.—Bottom: O_2 uptake during 20 min. of recovery after exercise: mean values from twenty-two observations. Regression line and 95 per cent confidence limits shown for the first five points. The first point represents rest.

●——● Alveolar CO_2 tension during sixth and seventh minutes of exercise: mean values from twenty-nine observations.

—— Mean heart rate during sixth to tenth minutes of exercise ("plateau rate") after correction for "*Ermüdungsansteig*".

○——○ "Pulse deficit" as defined in text.

– – – – Acceleration of heart between the end of the first half-minute and end of fifth minute of exercise. Abscissa: O_2 uptake during steady state of exercise (fifth and sixth minutes).

Our results suggest that a rising body temperature may be responsible for cardiac acceleration after 5 min. exercise in the steady state. Knowleton and Starling [1912] and Badeer [1951] found a linear relation between, on the one hand, the temperature of venous blood or of the sino-atrial node and, on the other, the rate of isolated heart preparations in cats and dogs. Hellerstein and Liebow [1950] were able to vary the heart rate by varying the temperature of a heating device placed in the right atrium. An increase in temperature of mixed venous blood may thus exert a direct effect upon the pacemaker. Christensen [1931], on the other hand, could find no relation between the heart rate and the body temperature during exercise. His experiments were different from ours in several respects (rectal thermocouple, less frequent pulse counts by palpation of the carotid artery, exercise up to 1 hr. on a bicycle ergometer) and it is not possible to explain the discrepancy between our results and his on any other basis.

Müller's correlation of the "fatigue-rise" with the state of training is compatible with the temperature hypothesis if one assumes that a fit subject loses heat more rapidly than an unfit subject of the same build under the same conditions of energy expenditure and of environmental temperature, air-flow and humidity. There is no proof that this is the case during submaximal work in ordinary surroundings. Alternatively, the explanation may lie in the facts that Müller's experiments were done on a bicycle ergometer (a form of exercise markedly subject to variation in skill, as shown by Erickson *et al.*, 1946) and that energy expenditure was expressed as calculated external work in mkg./sec. If by an "untrained" subject we understand a subject unaccustomed to bicycle work it may well be that such a subject expends more energy in performing the same amount of external work. Inefficient exercise might thus result in greater heat production and a bigger "fatigue-rise" in heart rate. The only means of settling this point is to measure oxygen consumption during work, and this was omitted in Müller's study.

Our findings with regard to oxygen debt are open to criticism on the ground that a 20-min. collection would not cover the larger debts. Nevertheless, it provides a relative measure of debt which is adequate for the present purpose. The debt curve in fig. 4 is very similar to that of Margaria *et al.* [1933] who found that in treadmill exercise the end of the rectilinear phase at an O_2 uptake of about 2000 ml./min. corresponded with the "alactic-lactic" threshold. Although blood lactate was not estimated in our experiments the behaviour of the alveolar CO_2 tension (fig. 4) suggests that an O_2 uptake of 2000 ml./min. marked the beginning of a metabolic acidosis. It will be recalled that alveolar air was sampled as soon as the steady state of lactate concentration in the blood could be assumed [Asmussen, 1950] and before the rise in body temperature could have had a major effect upon pulmonary ventilation. That a rise in temperature was not responsible for the depression in alveolar P_{CO_2} is further suggested by the fact that above an O_2 uptake of 2000 ml./min. there was no further rise of temperature (fig. 3) whereas alveolar P_{CO_2} continued to fall. We think it reasonable to assume that the point of departure of the debt curve from a straight line and the summit of the alveolar P_{CO_2} curve indicate a level of effort at which acid metabolites began to increase substantially in the circulating blood.

As shown in fig. 4, neither the absolute heart rate in the steady state of exercise (after correction for the presumed effects of change of temperature) nor the rise in heart rate from the end of the first half-minute to the steady state value undergo any apparent change in trend during the transition from "alactic" to "lactic" levels of exercise. This does not support the idea that the chemical factors involved in this transition are directly responsible for increasing the heart rate through a chemoreflex, a possibility suggested by Aviado and Schmidt [1955]. On the other hand, the "pulse deficit" of early exercise becomes appreciable only when the rate of work exceeds the supposed alactic-lactic threshold. The significance of this coincidence is obscure but it would seem likely that the "pulse deficit" (by analogy with the oxygen deficit of early exercise) is associated with factors which determine lactate production and lactic oxygen debt, rather than that lactate concentration determines the pulse deficit. Beyond this we are not prepared to speculate at present.

Our results indicate, however, that of all the criteria to be derived from the response of the heart rate to exercise, the "pulse deficit" of early exercise is the only one which bears a relationship to the "alactic-lactic" threshold. This threshold may well be one of the very few reliable indices of an individual's capacity for exercise, but its determination is far too tedious for routine application. The heart rate during recovery from standard exercise has proved a poor index of a subject's capacity for exhausting exercise [Taylor, 1944] or for a variety of athletic and gymnastic performances [Cureton *et al.*, 1945]. It would be interesting to see whether the "pulse deficit" of early exercise would provide a better criterion.

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THE ACTIVITY OF PULMONARY STRETCH RECEPTORS DURING CONGESTION OF THE LUNGS.

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The effect of pulmonary congestion on the activity of "slowly adapting" pulmonary stretch receptors has been investigated in cats with closed chests. During positive pressure inflation of the lungs congestion caused a mean increase in discharge rate of about 20 per cent at any volume; this was more uniform with large inflations. A similar sensitization was seen when cats were breathing spontaneously or by means of a phrenic stimulator, and in spontaneously breathing cats the Hering-Breuer inflation reflex was enhanced. The receptors probably lie in the walls of the air-passages, and congestion will increase the degree of stretch of these walls, since alveolar compliance is reduced with a greater pull on the airways. The reasons why previous workers have had negative results is discussed, together with the possible rôle of pulmonary stretch receptors in the respiratory changes caused by pulmonary congestion.

RECENT work on pulmonary stretch endings indicates that they do not behave as straightforward lung volume receptors, as believed by Adrian [1933]. Although their primary stimulus is the volume of the lungs, their response at any lung volume is influenced by bronchial tone [Widdicombe, 1954], by transthoracic pressure [Weidmann and Bucher, 1951] and by the previous history of the lung [Davis, Fowler and Lambert, 1956]; on the other hand Bülbring and Whitteridge [1945] have shown that their activity is not affected by vascular congestion in perfused cats' lungs. This last result is surprising, both on theoretical grounds and in the light of the recent work mentioned above, although it is not easy to interpret in the absence of definite evidence for the site of the end-organs. In the cat, the pleura has been eliminated, and indirect evidence suggests that many of the end-organs lie in the air passages rather than the alveoli [Davis *et al.*, 1956: Widdicombe, 1954]. In either site they should be affected by the degree of pulmonary congestion. In the alveolar wall there would be a close relationship between capillary diameter and the shape of an end-organ, while pulmonary congestion should influence bronchiolar and bronchial mechano-receptors in so far as changes in the mechanical properties of the alveoli would alter gas volumes and mural tensions in the air passages. To investigate this problem further we have repeated the experiments of Bülbring and Whitteridge in cats with closed chests.

METHODS

Each cat was anæsthetized with pentobarbitone sodium (32 mg./kg.) intraperitoneally, and tracheal and venous cannulæ inserted. The animal's chest was opened on the left between two ribs during artificial ventilation of the lungs. The pericardium was opened and the cat heparinized (10 mg./kg.). A balloon (up to 2×3 cm. when distended) attached to polyethylene tubing was tied into the left atrium, and a polyethylene cannula for recording left atrial pressure was inserted on the pulmonary side of the balloon. On distension of the balloon the tip of the cannula probably lay in one of the pulmonary veins. The chest was closed after inserting a third cannula into the intrapleural space; this tube was used to empty the space and to record intrapleural pressure. After closure of the chest the cat breathed spontaneously. An alternative method for causing pulmonary congestion was to put a thread round the aorta; on tightening the thread through a polyethylene tube the vessel was constricted.

Distension of the balloon with 1–5 ml. of water raised the left atrial pressure to 20–40 cm. H_2O, and for any given balloon volume the level was reproducible. Pressure was never maintained for more than 2 min., and was usually restricted to 0·5 min. to lessen the risk of pulmonary œdema. The latter was never seen. Constriction of the aorta caused larger changes in left atrial pressure (up to 80 cm. H_2O) and a profound fall in systemic blood pressure. Since reproducible and moderate changes could not be obtained by this latter method it was only used in five cats. In these a similar loop was put round the pulmonary artery to compare the effects of vascular obstruction with and without pulmonary congestion during spontaneous breathing. It was not used while recording activity in pulmonary stretch fibres.

Left atrial, intrapleural and, in some cats, femoral arterial pressures were measured by capacitance manometers attached to saline-filled cannulæ. The frequency responses of the units with cannulæ attached were 150–200 cycles/sec. Intratracheal pressures were measured with an air-filled capacitance manometer with a considerably better frequency response. Tidal volumes were usually measured by integrating the electrical output of a gauze-screen differential-pressure pneumotachygraph; this was similar to but proportionately smaller than that described by Lilly [1950] for man. Since the integrating circuit had a time constant of 40 sec. it was unsuitable for measuring small (0–10 ml.) changes in functional residual capacity. In some experiments, therefore, tidal volume was measured by attaching the tracheal cannula to a 60 l. air reservoir in which the pressure changes were measured with a capacitance manometer. These pressure changes were always less than 1 cm. H_2O.

Action potentials in single active nerve fibres of the right cervical vagus were recorded by dissecting small strands from the nerve trunks and placing them on saline-wick electrodes. The left vagus nerve was not used since the left lung might be damaged by the thoracic operation. A conventional R.C. amplifier was used with cathode-ray tube and camera. The response of pulmonary stretch receptors to positive pressure inflations was followed either by ventilating the lungs with a constant volume respiration pump, the cat being slightly hyperventilated to inhibit spontaneous respiration, or by arresting the pump and inflating the lungs with a syringe. The latter method allowed lung-volume/discharge-frequency curves to be drawn. Impulse frequencies were measured at the peak pressure on pump inflation, and 1 sec. after completion of a volume change on syringe inflation. Negative pressure inflations of the lungs were achieved either by allowing spontaneous respiration or by using a phrenic stimulator. This was of conventional design for clinical use, and its output was connected to the uppermost roots of the phrenic nerves on both sides using silver electrodes. The cats were slightly overventilated. The tidal volumes usually progressively decreased during phrenic stimulation at constant strength.

The Hering-Breuer inflation reflex was investigated in spontaneously breathing cats by inflating the lungs with a known volume of air (40–100 ml.). The intrapleural pressure record indicated the length of inhibition of breathing, and this was expressed as a ratio of the respiratory interval during the control period before inflation. This arbitrary index was reproducible under uniform experimental conditions [May and Widdicombe, 1954].

The cervical vagi were cooled by placing them on silver "thermodes" through which cooled alcohol was circulated; the apparatus was similar to that described by Dawes, Mott and Widdicombe [1951].

RESULTS

The easiest way to investigate the effect of pulmonary congestion on stretch receptor activity is to ventilate the lungs with a constant volume pump and to record changes in peak discharge rate at different pulmonary vascular pressures. This was the method used by Bülbring and Whitteridge [1945] on perfused lungs. We found that in cats with closed chests distension of a balloon in the left atrium caused maintained and reproducible rises in pulmonary venous pressure to 20–40 cm. H_2O. Eight receptors were investigated in this way (Table I) one of which is illustrated in fig. 1. Although all the end-organs showed increased discharge on pulmonary congestion, this could have been due to an increase in functional residual capacity. Since the functional residual capacity was not increased by more than 5 ml. when the left atrium was obstructed during spontaneous breathing (range 0–4·8 ml., in four cats during fourteen periods of congestion; these values agree closely with those of Bülbring and Whitteridge) and occasionally by up to 10 ml. when the aorta was constricted (range 2·5–10·1 ml. in two cats, four periods of congestion), it seemed unlikely that the increases in discharge were due to uniform rises in lung volume. For six receptors in Table I correction has been made assuming an increase in functional residual capacity of 5 ml. From the volume/discharge curves of these endings (see below) the rise in discharge frequency corresponding to a 5 ml. increase in lung volume was measured and subtracted from the rise in frequency due to congestion. Of these receptors four showed significant ($P < 0\cdot01$) increase in impulse frequency after this correction had been made. It remained possible, however, that there was a change in the distribution of gas in the lungs, and that the receptors lay in pulmonary units which were relatively overdistended.

Table I.—The Effect of Pulmonary Vascular Congestion on the Peak Frequency of Discharge of Stretch Receptors stimulated by Constant Volume (30–50 ml.) Inflations of the Lungs with a Respiration Pump. The Results are Averages of Five Estimations before, during and after Congestion (total fifteen). Standard Errors of the Means are given. The Significances of the Changes in Impulse Frequency corrected for a 5 ml. Increase in Functional Residual Capacity are given.

Receptor	Peak frequency of discharge (impulses/sec.)				Significance P
	Before and after congestion	During congestion	Change during congestion	Change corrected for 5 ml. rise in volume	
1	125 ± 2·5	166 ± 2·9	+41	+33	< 0·01
2	134 ± 1·7	148 ± 3·2	+14	+6	< 0·1
3	81 ± 0·8	90 ± 1·6	+9	+6	< 0·01
4	94 ± 5·4	105 ± 3·6	+11	+8	< 0·1
5	64 ± 1·7	79 ± 1·7	+15	+12	< 0·01
6	95 ± 0·5	120 ± 0·7	+25	+20	< 0·01
21	96 ± 0·8	119 ± 1·1	+23	..	..
22	17 ± 1·4	20 ± 2·2	+3	..	..

A more thorough method of analyzing receptor activity was used in plotting volume/discharge frequency curves before and during pulmonary congestion over a wide range of volumes. Inflations were made with a syringe, and eighteen receptors were examined. Fig. 2 shows a record from one end-organ, and fig. 3 illustrates responses from three receptors. Thirteen endings were unequivocally sensitized throughout the range of inflations (fig. 3, C), although in five of these the change was relatively small, under 15 per cent increase in impulse frequency (B). Sometimes the effect was greatest at large volumes, sometimes at small. Three receptors showed an increased discharge with larger inflations but a slight effect or no change with small inflations (A). One receptor had a decreased discharge at all levels of inflation and one was not measurably affected by congestion. In an attempt to express these results quantitatively Table II has been prepared, which shows the change in discharge frequency at small and at large inflation volumes. It will be seen that in the former instance there is a slightly smaller mean change with rather greater variation than with large volumes. With both volume ranges the mean difference in discharge frequency is significantly above zero ($P < 0{\cdot}01$). In determining the significance of the change in impulse frequency corrections should be made for the increases in functional residual capacity caused by congestion. However it proved impossible to measure these accurately during inflations of the lungs with a syringe. We

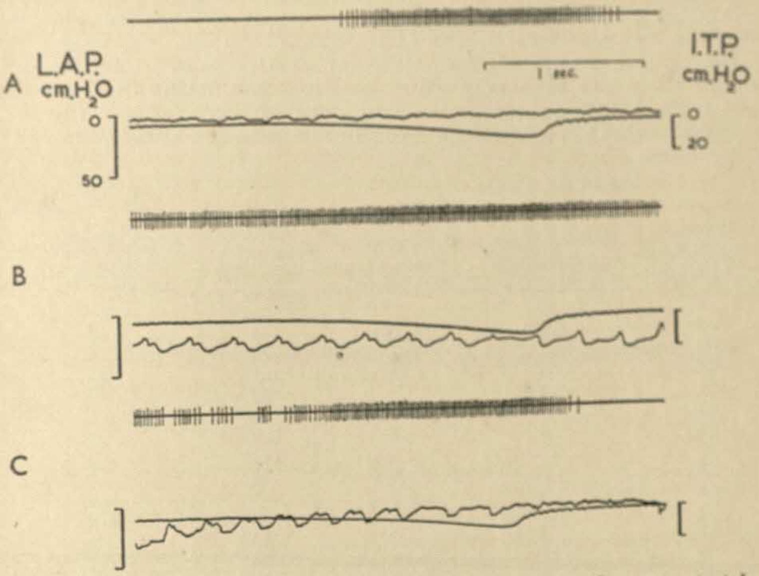

Fig. 1.—Effect of pulmonary vascular congestion on a pulmonary stretch receptor during phasic positive pressure ventilation from a pump (40 ml.). Uppermost trace: action potentials in a single vagal fibre. Middle trace: left atrial pressure (positive downwards). Lowest trace: intratracheal pressure (positive downwards). A, before; B, during and C, after pulmonary congestion. During congestion there is a greater peak frequency, and the receptor fires during expiration. Action potentials retouched.

have, therefore, once again assumed that the functional residual capacity might have increased by 5 ml. (the greatest increase seen when congestion was caused by left atrial obstruction). On this basis the figures in Table II have been corrected. Since the values for "large inflations" were one step below the maximum inflation, this has involved interpolation but not extrapolation of the curves. From these results it seems that even an increase in functional residual capacity of 5 ml. would not account for the sensitization obtained. In Table II standard errors of the means are not included for individual receptors, since usually only one or two inflations with a syringe were done during congestion for each end-organ. However each inflation included measurements at five to six intermediate volumes, as shown in figs. 2 and 3.

Table II.—The Effect of Pulmonary Vascular Congestion on the Frequency of Discharge (measured 1 sec. after each Inflation) of Stretch Receptors stimulated by Inflations of the Lung with a Syringe. Standard Errors of the Means are given for the Changes in Discharge Frequency. Large Inflations are one Inflation Step less than the Maximum Volume for any given Cat (50–125 ml.); Small Inflations are about one-third of this (20–50 ml.). Results corrected for an Increase in Functional Residual Capacity of 5 ml. are included.

	Small inflations			Large inflations		
Receptor	Before and after congestion (impulses/sec.)	Change during congestion (impulses/sec.)	Corrected change (impulses/sec.)	Before and after congestion (impulses/sec.)	Change during congestion (impulses/sec.)	Corrected change (impulses/sec.)
2	96	+15	+9·5	172	+43	+24·5
3	62	+2	−3	78	+14	+10
5	24	+32	+24	61	+15	+13
6	101	+15	0	132	+10	+7
7	81·5	+5	+3	94	+4	+3·5
8	89·5	−3	−3	100	+2·5	+1·5
9	92	+4	+1·5	121	+9	+6
10	36	+5	+2·5	75	+10	+5
11	54	+4	+2	75	+6	+4·5
12	33	−2	−5	63	−8	−12
13	19	+4	+2	44	+5	+3·5
14	39	+11	+10	55	+7·5	+6·5
15	57	−3	−4·5	69	+1·5	+1
16	60	+15	+13	75	+17	+15·5
17	22	+60	+54·5	69	+32	+29
18	58	+29	+27	90	+39	+37
19	9·5	+27	+24	43	+14	+11·5
20	58	+8	+6	85	+8	+5·5
Means	55	12·1 ± 3·8	9·1 ± 3·5	84	12·8 ± 3·1	9·6 ± 2·6
Significance (P)		< 0·01	< 0·05	..	< 0·01	< 0·01

We have tried to discover if pulmonary stretch receptor activity was influenced by pulmonary congestion during spontaneous respiration. This is difficult to do since the tidal volume is altered, and only if the tidal volume is unchanged or reduced and the receptor discharge is increased by congestion can a sensitization be claimed. Of five receptors investigated three behaved in this way, the other two being equivocal. However the cats breathed more rapidly as well as more shallowly, and it is possible that a quick inflation

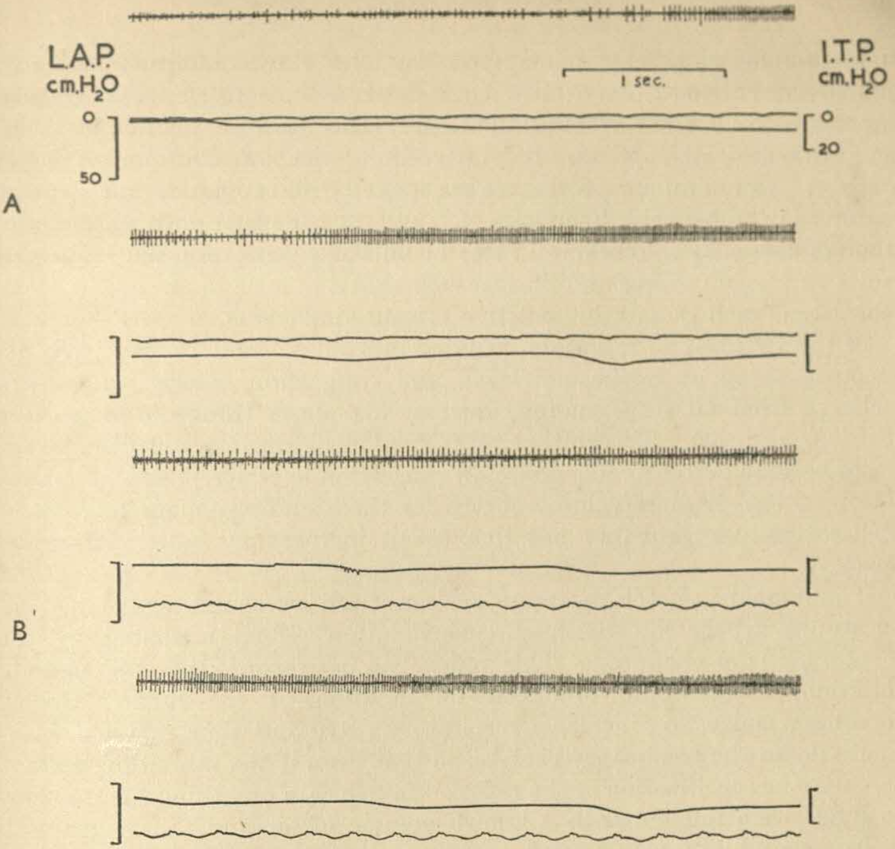

FIG. 2.—Sensitization of a pulmonary stretch receptor by pulmonary congestion. The lungs were inflated with a syringe in steps of 20–100 ml. total. Traces as in fig. 1. A, before congestion; B, during congestion. Action potentials retouched.

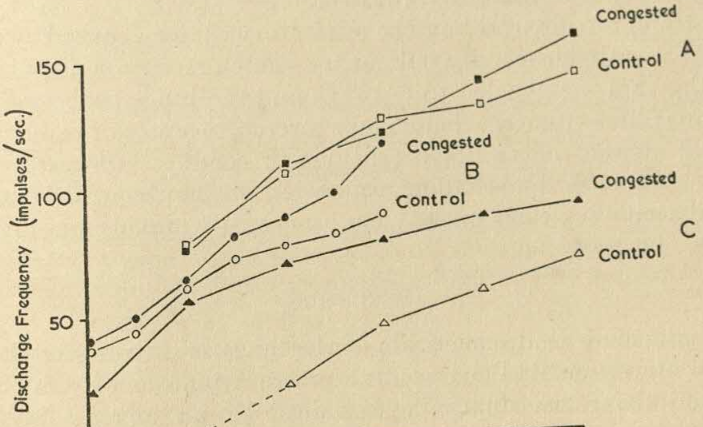

FIG. 3.—Inflation-volume/discharge-frequency curves for three receptors, before and during pulmonary vascular congestion.

would produce as great a peak frequency as a slower inflation to a larger volume, since the receptors (although "slowly adapting") have a significant adaptation to constant volume inflations. The use of a phrenic stimulator should obviate this objection, but for technical reasons we found it difficult to apply. Only two receptors were successfully investigated, and both gave greater peak frequencies (increases of 6 and 13 per cent) on lung congestion although the tidal volumes and rates of inflation were reduced. These few results with spontaneous and phrenic stimulated breathing are not, therefore, inconsistent with those using positive pressure inflations.

Of the twenty-two receptors included in Tables I and II, five were spontaneously firing at expiratory level, and congestion caused an increased discharge from all these endings during this phase (range 6–66 per cent; mean 25 per cent). Three other receptors, silent during control expiratory pauses became active at this phase on congestion (*e.g.* figs. 1 and 2). Examination of the pressure/volume curves for these endings suggested that the sensitization was probably not due to an increase in functional residual capacity.

If pulmonary stretch receptors are sensitized by congestion of the lungs one would expect the Hering-Breuer inflation reflex, mediated by these receptors, to be enhanced. This reflex can be recorded as the period of inhibition of breathing produced by inflation of the lungs. However pulmonary congestion increases respiratory drive and thus sets a new level of activity in the respiratory centres, so that even if the inhibition is shorter there may be sensitization of the reflex. In five cats breathing spontaneously the lungs were inflated with a known volume of air and the length of the respiratory inhibition measured and expressed as a ratio of the previous respiratory interval. In four of these cats the ratio was consistently increased by congestion caused by obstruction of the aorta, often with an absolute increase in the time of inhibition (fig. 4).

In an attempt to discover how the respiratory changes caused by congestion of the lungs were influenced by pulmonary stretch receptors, in three of these five cats the vagi were cooled to 8–10° C. and the lungs congested. At this temperature fibres from the pulmonary stretch receptors are blocked while other vagal afferent nerve fibres continue to conduct [Dawes *et al.*, 1951]. With the vagi cooled rapid shallow respiration was not seen, although respiratory stimulation was clearly present, the cats usually making long and powerful inspiratory efforts (fig. 5).

DISCUSSION

Our results show that congestion of the lungs sensitizes stretch receptors when they are stimulated by positive pressure inflations from a pump or syringe, and that this cannot be explained by an increase in functional residual capacity. More reliance is to be placed on the results with inflations with a syringe, since pulmonary stretch receptors are influenced by the rate of inflations [Davis *et al.*, 1956] as well as by lung volume. Using a syringe, discharge rates were measured 1 sec. after inflation was complete, *i.e.*

under nearly static conditions. Some end-organs gave far higher frequencies when stimulated by pump inflations of 30–50 ml. than when equivalent volumes of air were injected by a syringe. This is due to the receptors responding to the active phase of inflation as well as to the maintained inflation. It is

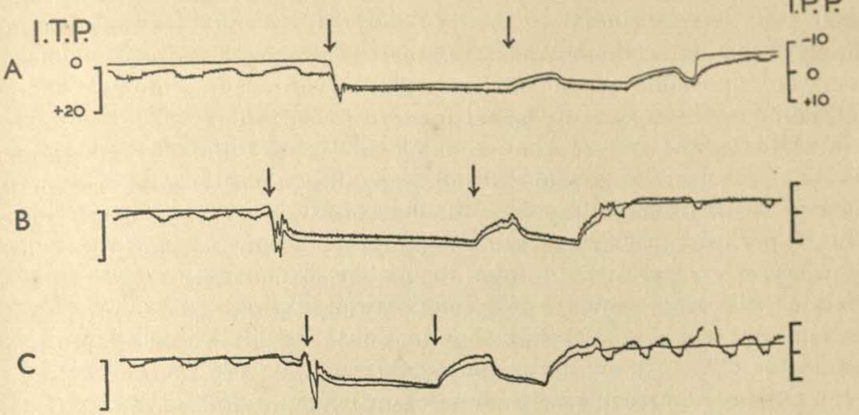

Fig. 4.—Changes in the Hering-Breuer inflation reflex caused by constriction of the aorta (B) and constriction of the pulmonary artery (C). A is a control. Upper trace, intratracheal pressure (positive downwards). Lower trace, intrapleural pressure (positive downwards). The length of respiratory inhibition is indicated by the two arrows on each record. In B, although there is stimulation of breathing caused by the aortic obstruction, there is a longer inhibitory pause. In C there is also respiratory stimulation, but no potentiation of the reflex. The inhibitory ratios (see text) for the three records are 300, 455 and 253 per cent respectively.

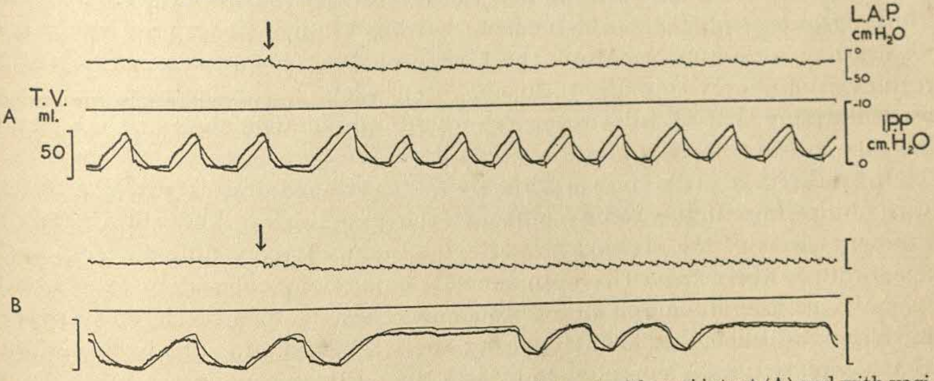

Fig. 5.—Effect of pulmonary congestion on respiration in a cat with vagi intact (A) and with vagi cooled to 8° C. (B). With the vagi cooled there is no rapid shallow breathing, but prolonged inspirations are present. Uppermost trace: left atrial pressure (positive downwards). Middle trace: tidal volume (inspiration upwards). Lowest trace: intrapleural pressure (positive downwards). Congestion is started at the arrows.

unlikely that the rises in discharge rate on congestion were due to increase in functional residual capacity. The results remained statistically significant even assuming an increased volume of 5 ml., and the mean increase in lung volume which would cause equivalent increases in discharge frequency was over 20 ml.

At first sight these results seem to contradict those of Bülbring and

Whitteridge [1945] who used a perfused lung preparation. However in Table II it will be seen that with moderate volumes of inflation three receptors showed a decreased rate of discharge on congestion of the lungs, and that the mean increase is considerably influenced by four receptors (5, 17, 18 and 19) which were greatly sensitized. Apart from these four our results at this level of inflation are similar to those of Bülbring and Whitteridge in giving a variable response which might be explained, as they suggest, by unequal ventilation of pulmonary units consequent to vascular congestion. With large volumes, however, we found a more consistent sensitization and the mean increase was greater than that at smaller volumes. With the larger inflations the effect of unequal ventilation would be minimized, although these inflations (with trans-pulmonary pressures of 10–15 cm. H_2O) would be well within the vital capacity of the cat. In preparations of perfused lungs one might expect greater distributional inequality of ventilation than in animals with closed chests; and if the lungs were allowed to collapse between inflations this would both reduce the functional residual capacity and increase the number of poorly ventilated pulmonary units. We therefore think that our results are compatible with those of Bülbring and Whitteridge, but that by working under more physiological conditions and by using a greater range of inflations the sensitization of pulmonary stretch receptors by congestion of the lungs has become apparent.

The sensitization of the end-organs is presumably due to the greater stretching of the air passages when the compliance of the alveoli is reduced. This assumes that the airways are the site of the receptors [Widdicombe, 1954; Davis *et al.*, 1956]. The fact that the change in impulse frequency was not greater must rule out the alveoli as their localization, which is in any event unlikely since they do not often adopt a cardiac rhythm. It is most unlikely that alveolar receptors would only change their discharge rate by 20 per cent when pulmonary capillary pressure rises to 40 cm. H_2O. The variability of our results is probably due to two factors; left atrial pressure was not raised to the same value in each experiment, and end-organs in different parts of the airways would show quantitatively different responses according to their exact site. In the cat, pulmonary congestion causes only a small decrease in compliance whether the chest is open or closed [Hughes, May and Widdicombe, 1958]; raising the left atrial pressure to 20–40 cm. H_2O decreased static compliance by less than 10 per cent in the majority of experiments and this may explain the small size of the sensitization seen.

The extent to which the sensitization may influence the respiratory response to congestion is difficult to assess. The few experiments with spontaneous and phrenic stimulated breathing suggest that receptors are also sensitized under these conditions. It is probable that the Hering-Breuer inflation reflex is enhanced by congestion of the lungs, but the absence of a satisfactory method of measuring the potency of the reflex under conditions of changing activity in the respiratory centres makes this conclusion tentative. With the vagi cooled to block the afferent nerve fibres from the stretch receptors, pulmonary engorgement caused maintained inspiratory efforts rather than rapid shallow breathing, so the rôle of sensitized end-organs may be to convert the former pattern to the latter. This would be expected from their physiology.

Whitteridge and Bülbring [1944] have shown that sensitization of pulmonary stretch receptors by volatile anæsthetics produces rapid shallow breathing. In their experiments a sensitization of 100–200 per cent or even more only reduced tidal volume by 50 per cent, so a sensitization of 20 per cent by pulmonary congestion should exert a far smaller effect. There has, however, been little quantitative work on the effect of impulses from pulmonary stretch receptors on the respiratory cycle.

There is good evidence that congestion of the lungs influences respiration by afferent pathways other than that of the stretch fibres (see Whitteridge [1950]; Aviado and Schmidt [1956], for references). In our experiments respiratory stimulation was produced by pulmonary congestion when the stretch fibres were blocked, and there was also evidence that back pressure effects proximal to the lungs contributed to the respiratory changes in the intact animal (*e.g.* fig. 4, C). Therefore although our results support Christie's [1938] view that increased stiffness of the lungs should sensitize pulmonary stretch receptors, we have no wish to revive the theory that this sensitization is responsible for the dyspnœa and rapid breathing of clinical pulmonary congestion; it is merely thought that increased activity from these endings may be one factor in the altered breathing caused by congestion of the lungs. A further reason for caution is the danger of transferring results from one species to another, especially since there is evidence that the Hering-Breuer reflexes are very poorly developed in man compared with the cat and other species [Marshall and Widdicombe, 1958].

ACKNOWLEDGMENTS

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BOOK REVIEWS

THE CLINICAL APPLICATION OF HORMONE ASSAY. By J. A. LORAINE.

Edinburgh and London: E. & S. Livingstone, Ltd. 1957. Pp. xii + 368. 30s.

This book is really about "Hormone Assays for Clinical Application". Its main contribution is a much-needed review of most of the clinically applicable assays of urinary or blood hormonal level. Dr. Loraine gives a readable outline of this developing subject, and without wearisome details makes clear the limitations and potentialities of the methods. He is particularly at home, of course, in expounding the Edinburgh views, and in recounting the experiments and the clinical experience gained with these methods at the Edinburgh Clinical Endocrinology Research Unit. The book is particularly useful for its chapters covering the assay methods recently studied and often developed there; especially the assays of urinary gonadotrophins, œstrogens, pregnanediol, and also of adrenaline and noradrenaline.

The introductory chapter is on the requirements for a satisfactory bioassay, and on the criteria for determining the accuracy, precision, specificity and sensitivity of any assay. Previous investigators have not always followed these precepts, as the reader is rather frequently reminded in the rest of the book. Many readers might have liked all the normal values for the recommended assays assembled together, perhaps at the end of this introductory chapter.

Then follows a series of chapters on the various hormones which have been assayed in human blood and urine. The assays of established clinical validity and value, such as those of the pituitary gonadotrophins, chorionic gonadotrophin, œstrogens, pregnanediol, corticosteroids, 17-ketosteroids, and adrenaline and noradrenalin might well have been segregated into a separate section of the book. But they appear *seriatim* along with others of potential clinical value such as the thyrotrophin and insulin assays, and others still needing development before they can reach this stage—*i.e.* ACTH, prolactin, growth hormone and antidiuretic hormone.

Each chapter first covers the methods of extraction and then those of the assay, finally reviewing the normal values found, and then the findings in various disease conditions. There is an excellent account of the normal variations in gonad and gonad stimulating hormones, including those of the menstrual cycle and pregnancy; and this book should stimulate the gynæcologist and the clinical endocrinologist to define more practical uses for these assays in menstrual and allied disorders. The studies of chorionic gonadotrophin levels in pregnancy toxæmia and in diabetic pregnancies are well reviewed, and the conclusion drawn that this assay has little practical value in these conditions. The chapters on the assay of insulin, ACTH and TSH are justifiably brief pending better methods; but the reader is introduced to the problem he would encounter in attempting these assays and to most of the main recent literature. The author has probably been wise to omit reference to the assay of thyroid hormone, since so many other methods are available for assessing thyroid secretory function; but this might have given Dr. Loraine an opportunity to assess the value of hormonal levels as an index of glandular secretory activity or of tissue supply of the hormone—an assumption basic to the use of most of these assays but not valid for all of them always. The corticosteroid chapter will disappoint any reader hoping to find the best assay method for indexing adrenal cortical function; but this probably reflects the imperfection of available methods and knowledge. Dr. Loraine might have been kinder to the reader if he had merely given historical recognition to certain superseded methods such as those for the reducing and formaldehydogenic steroids, and if he had treated the assay of aldosterone as a separate problem. For most of his readers, corticosteroids are the steroids involved in the secretion of the adrenal cortex's main hormone or cortisol. Some clinicians may have difficulty in recognizing "21-dihydroxy-20 ketocorticosteroids" as Porter-Silber steroids or Porter-Silber 17-hydroxysteroids. But the main methods of clinical value are covered, their chemical significance clarified and their relative value indicated.

This book is an essential reference volume for any laboratory or clinic performing endocrine assays or frequently using them. It should help them choose the best assay methods, and suggest to them where present experience permits clinical interpretations, and where further research is most needed. As a reference source for recent experience with any assay, physiologists, biologists, chemical pathologists as well as clinicians, should find it extremely helpful. The clinician can easily ascertain the published findings with any assay, but not how to use these assays to help him establish a clinical diagnosis; but it is probably too early for such a book. Many clinicians wondering what all these hormone assays are about should delve into this book, and thereafter we may expect them to make fewer airy requests for such assays.

T. Russell Fraser.

AN INTERNATIONAL SYMPOSIUM ON ALDOSTERONE. Edited by A. F. Muller and Cecilia M. O'Connor. London: J. & A. Churchill, Ltd. 1958. Pp. x + 232. 40s.

The history of aldosterone is interesting for several reasons, one being the speed with which it has become available since its crystallization in 1953. Despite an immense amount of work since then, there is still a great deal we do not know about this substance which is essential to the life of man and of many animals. A symposium on aldosterone was therefore important for those working in the field, and now makes interesting reading for others. This hormone is remarkable for the fact that it is made in the adrenal cortex which is subject to anterior pituitary control, and yet is itself relatively free of direct control from that quarter. Yet some indirect pituitary control exists since Giroud, Stachenko and Pilletta found that growth hormone promoted an increase in urinary aldosterone in a boy with hypopituitarism, and that posterior pituitary extract contained an unknown factor which raised the output of aldosterone more than that of cortexone from slices of beef adrenals. They also confirmed that aldosterone is made specifically in glomerulosa cells. Bartter, Biglieri, Pronove and Delea found that expansion of the intravascular volume was followed by a decrease in aldosterone excretion and conversely contraction by an increase in aldosterone excretion. They therefore believe that the influence by Na on aldosterone secretion depends upon changes in intravascular volume.

A serious hindrance to the study of aldosterone is the lack of a simple method for its identification in any body fluid other than urine, and even here assay is a laborious process. Moreover, only about 2–3 per cent of the amount secreted by the gland appears in the urine. Thus the extraction and chromatographic method described by Moolenaar and Querido is of great interest. Another aspect of adrenal activity about which more information is needed is the qualitative differences between its various products of secretion; this was discussed by Desaulles, and by Gross and Lichtlen. A facet of this same problem is the normal human gland content of adrenal cortical hormones. Clearly this information is not easy to obtain, but Neher presented some figures from both normal human adrenals and those with tumours. The metabolic path of tagged aldosterone was followed [Ayres, Barlow, Barrod, Kellie, Tait, Tait and Walker] and it was found that aldosterone disappeared rapidly from blood and that in normal humans the urinary solvent fraction from which radioactivity was recovered varied according to the salt content of the diet. Calculation showed that the daily secretion of aldosterone is 190 μg. on a normal salt diet, and 780 μg. on a low one. This means that the aldosterone concentration of plasma is probably about 0·03 μg. per 100 ml. plasma, so that its estimation in blood is not likely to be a practical clinical procedure.

Amongst the clinical aspects of aldosterone discussed was the diurnal variation in its production with relation to position and activity in normal and hypopituitary subjects [Muller, Manning and Riondel]. Unlike the diurnal variation in 17-hydroxycorticoids, that of aldosterone is not constant, since inactivity and the horizontal position abolish it. This is further evidence that the control of aldosterone output, as well as its significance, is different from that of the 17-hydrocorticoids. The diurnal variation in aldosterone secretion is not seen in hypopituitary subjects, even when they are ambulatory and active. Ayres, Garrod and Tait discussed primary aldosteronism and offered suggestions for the absence of œdema despite Na retention, and contrasted this with the state of affairs in secondary aldosteronism where œdema is present although there is less disturbance of K distribution. Stanbury, Gowenlock and Mahler contributed a paper on the interrelationships of K deficiency and renal disease. The last three papers of the symposium were given to the consideration of œdema formation and its relationship to cortexone [Stahl, Stephan, Jahn, Urban and Jahn], hyperaldonsteronuria [Mach] and the aldosteronuria which may be seen in œdema [Wolff, Kozcorek and Buchborn]. The discussions are printed and give a most interesting insight into the ideas and views of the speakers. The book is a valuable mine of information and references on a new, important and rapidly expanding subject.

MARY PICKFORD.

IMMUNOPATHOLOGIE IN KLINIK UND FORSCHUNG. Compiled and edited by Dr. P. Miescher and Dr. K. O. Vorlaender. Stuttgart: Georg Thieme. 1957. Pp. xv + 598. D.M. 69.

This handsome volume is in the best tradition of German publication. Under the general editorship of Dr. Miescher and Dr. Vollaender, a most distinguished group of contributors (from the Pasteur Institute in Paris, from Berne, Basle, Freiburg, Munich and Bonn) have presented a vast amount of information in a most readable fashion. The wide range of subjects covered includes the theoretical basis of immunity, anti-erythrocytic antibodies, the immunology of leukocytes and blood platelets, of renal and hepatic disease, and of rheumatic and encephalomyelitic reactions. Essentially the text falls into two sections, the first and smaller dealing with the theoretical basis of immunity under each of these headings; the second and major portion of the work is concerned with the practical application of immunological techniques in the investigation and treatment of patients. In this clinical section all the growing points of the subject are considered, as for example the hæmolytic anæmias and purpura, diseases of the thyroid, L.E. phenomenon, organ transplantation and sympathetic ophthalmia.

There is no doubt that this book is an important contribution, and will form a landmark against which to measure a rapidly changing field of medicine. The bibliography is excellent, but it must be emphasized that the text is in no way a compilation; each author has a personal experience which ensures a critical approach and gives the stamp of authority to his opinions.

Geo. L. Montgomery.

VERGLEICHENDE NEUROPATHOLOGIE DES MENSCHEN UND DER TIERE. By E. Frauchiger und R. Fankhauser. Berlin, Göttingen, Heidelberg: Springer. 1957. Pp. viii + 451. D.M. 126.60.

This interesting book is concerned with spontaneously occurring disorders of the central and peripheral nervous systems (and of muscle) of vertebrates—although the endocrine system and brain of the bee also receive mention. The aim of the book is "to give an account of the present position of animal neuropathology and to fit it—but not to force it—into the framework of human neuropathology", and this it does well.

The book is partly based on an extensive study of the literature of many languages, but mainly on personally studied post-mortem material of 2900 cases, all with clinical histories. Many of the animals were examined by the authors during life and, rather than relying on material sent to them, the authors performed the autopsies themselves in the majority of cases—which in large animals and often under difficult conditions represents an immense labour—and this greatly enhances the value and reliability of their observations. The subjects covered include short chapters on comparative anatomy and physiology, with data such as the composition of cerebrospinal fluid in different species. The whole range of neuropathological problems is discussed, from congenital malformations through infections and degenerative processes to trauma and neoplasms. There are numerous excellent macro- and micro-photographs.

Unfortunately the index is inadequate and difficult to use for the non-expert which must include many potential readers. "Scrapie", for example, occurs in two important contexts but only one is referred to in the index and there is no cross reference in the text. It is probably too much to ask but it would be of great help if German-writing authors of standard textbooks—and the book under consideration is certainly one of these—were to make the index with a thought for the foreign reader who may be inexperienced in continental terminology. For example, there are entries under Leucoencéphalite, Leukocencephalopathia, Leukoencephalitis, Leukoencephalopathie, Leukoencephalose and Leukodystrophie, but these diseases are not referred to under any other heading in the index. It is thus not easy to make full use of the valuable information contained in this book.

SABINA STRICH.

THE MAMMALIAN CEREBRAL CORTEX. By B. DELISLE BURNS. Monographs of the Physiological Society, No. 5. London: Arnold. Pp. vii + 119. 21s.

Most of our knowledge of the cerebral cortex is anatomical, even where derived by physiological techniques. It charts the motor areas and the receiving areas for impulses from the different sensory surfaces at the periphery, and describes the subdivisions of localization within these areas. Of its physiology, on the other hand, "it is probably wise to admit", with the author of this monograph, "that less is known about the functions of normal cerebral cortex than any other organ in the body". Horizontal spread of excitation and inhibition, whereby different regions may become linked in temporary or more permanent fashion, has obvious importance as a starting-point for study. At a time when movement of muscle was the only available measure of cortical action, Graham Brown succeeded in getting evidence of spread in the special case of the motor area. Generalization was achieved in Adrian's discoveries of the "surface negative" and "surface positive" electric responses of the cortex to local stimuli. This work has been continued by Delisle Burns, using a preparation of cortex isolated from nervous connexions but with preserved blood supply. In his monograph he discusses, in five essays intended to interest non-specialists, the general setting of his experiments. The first describes the preparation. The next discusses the nature of after-discharges, illustrating this with results mainly from other tissues. The next considers whether spontaneous neuronal firing occurs or not. The next surveys horizontal transmissions within the cortex. The last, "The Problem of Memory", looks at the difficulties confronting the neurophysiologist when he attempts to find what sort of enduring changes could be impressed on the brain in learning. Modesty in the face of these difficulties is a refreshing merit of this interesting little book.

C. G. PHILLIPS.

METHODS IN MEDICAL RESEARCH, Volume 7. Edited by J. V. WARREN. Chicago: The Year Book Publishers Inc. 1958. Pp. xiii + 237. $7.50.

This volume resembles its predecessors in consisting of a number of quite unrelated technical monographs. Each monograph has its own editor who usually writes most of it himself. This scheme has the obvious disadvantage that most of the book will be of no interest to any one reader. It also makes it difficult to review.

The contents of the present volume (with names of section editors) is as follows:

I. Chemical Investigation of Muscular Tissues [Mommaerts],
II. Hemodynamic Methods [Gorlin and Warren],
III. Methods for the Study of Human Leukocytes [Martin],
IV. Methods for Study of the Histology and Cytology of the Retina [Willmer].

The present reviewer is only competent to discuss the section on Hemodynamic Methods. This proved to be uneven, and on the whole disappointing. Considerable space is given to dilution methods of determining cardiac output, but the opportunity neglected of comparing the advantages of the various techniques. The longest chapter is on kinetocardiography, which is certainly not a method whose usefulness is established.

The value of previous volumes was enhanced by critical comments on the described techniques by independent workers. There appears to be only one such comment in the present volume.

A. C. DORNHORST.

ISOLE PANCREATICHE E GLUCAGONE. By C. CAVALLERO, B. MALANDRA and L. MOSCA. Societa Italiana di Endocrinologia, VII Congresso Nazionale. 1957. Pp. 283.

Recent advances in chemical physiology have reorientated interest towards cntracellular events. No longer can the biochemist close his eyes on cytology and iytochemistry by self dedication to the study of disintegrated cells and their derivatives. This monograph admirably synthesizes information from various fields to give a stimulating picture of the islet cells as active living units profoundly affecting the life of the animal as a whole.

The book is divided into three parts: the first two are devoted to the morphology and pathology of the islets, while the third is concerned with the function of glucagon.

The effects of age, endocrine activity and disease on the cytology of the islets are summarized with clarity. Elegant plates, mainly in colour, illustrate the authors' own important contributions to the subject. The factors, which affect the ratio of α- to β-cells in the islets, and also the selective injury to one or other of these two dominant kinds of insular cell are fully discussed. The authors conclude that it is doubtful if any other independent type of insular cell exists in the adult mammalian pancreas.

The origin of insulin from the granules in the β-cells is firmly established, and the less secure evidence for supposing that glucagon is derived from the α-cells is assessed. An account of work on the still uncertain rôle of glucagon forms much of the latter part of the book. Valuable summaries in Italian, English and German occupy the terminal twenty-four pages of text: these are followed by a bibliography of about a thousand separate publications. This excellent monograph, which forms so timely an addition to the literature of endocrinology, merits a more robust form of binding.

KENDAL C. DIXON.

HANDBUCH DER ZOOLOGIE. EINE NATURGESCHICHTE DER STÄMME DES TIERREICHES. Herausgegeben VON J. G. HELMCKE und H. v. LENGERKEN. Berlin: Walter de Gruyter & Co.

Delivery	Part	No.	Author	Title	Pages	Price D.M.
3	6	5	H. Mies	Physiologie des Herzens und des Kreislaufes	48	27
3	8	5	H. v. Hayek	Die Lunge	24	..
4	9	5	W. Schoedel	Die Atmung	96	36
5	2	5	F. Tischendorf	Milz	32	37.50
5	8	7	H. E. Voss	Der Einfluss endokriner Drüsen auf den Stoffwechsel der Säugetiere	70	..
6	8	4	C. Heidermanns	Physiologie der Exkretion	62	40
6	9	4	E. Heinz and H. Netter	Wasserhaushalt	46	..
7	3	10	G. P. Baerends	Aufbau des tierischen Verhaltens	32	42.75
7	21	10	P. Leyhausen	Das Verhalten der Katzen (Felidae)	34	..
7	5	5	H. Frick	Morphologie des Herzens	48	..
8	1	10	K. Lorenz	Methoden der Verhaltensforschung	22	28.50
8	6	10	I. v. Eibl-Eibesfeldt	Ausdrucksformen der Säugetiere	26	..
8	17	10	M. Meyer-Holzapfel	Das Verhalten der Bären (Ursidae)	28	..
9	10	10	K. Herter	Das Verhalten der Insektivoren	50	26.25
9	15	10	G. Tembrock	Das Verhalten des Rotfuchses	20	..
14	1	6	W. Krüger	Der Bewegungsapparat	81–176	42

These fourteen numbers of a comprehensive survey of the animal kingdom comprise part of the eighth and concluding volume. This volume will ultimately cover all aspects of the mammalian field in about one hundred numbers. The entire work has been in the course of production since 1925 and this final volume on Mammals is being produced after a gap of about fifteen years. Each delivery is apparently issued as soon as enough material is available and sometimes the juxtapositions of the numbers is not very convenient. However this can be corrected in a final binding when the volume is complete. This method of production in paper back covers with the final binding as the responsibility of the author is for reasons of economy, but even so the price of each delivery is high.

The numbers dealing with aspects of behaviour in such animals as the cat, fox and bear also cover the various techniques used to study behavioural patterns in a wide variety of animals. When complete, the account of this aspect of the life of mammals will comprise about a quarter of the whole volume.

The only aspect of physiology which is so far nearly complete is that covering the morphology and physiology of the heart and lungs. This is a valuable addition to comparative physiology and besides many good illustrations it has a number of useful comparative tables, for example heart rates, cardiac output, and frequency and volume of perspiration. Likewise in the number on excretion and water metabolism there are many tables of the various urinary constituents. The bibliographies are extensive and are divided into books and reviews and then the individual articles.

The numbers already published are sufficient to demonstrate that volume 8 when complete will be an invaluable book of reference for mammalian physiologists and zoologists. The printing is excellent and the illustrations (both photographs and line drawings) are up to the German traditions of excellence.

M. H. DRAPER.

BOOK NOTICES

LIGHT, COLOUR AND VISION. By Yves Le Grand. Translated by R. W. G. Hunt, J. W. T. Walsh and F. R. W. Hunt. London: Chapman & Hall. 1957. Pp. xiii + 512. £3, 3s. This learned work is aimed primarily at the physicist but it contains much information of value to anyone interested in the eye or in optical apparatus. Thus, whilst the first chapter deals with the concept of radiant energy, a physical problem, the last deals with "theories of the thresholds" and raises questions inevitably involving physiology. Particularly interesting is the review of "Anomalies of Colour Vision" (Chapter 15). Although this is only seventeen pages long it is a most useful summary of this field. How sensible it is to mention rather than to neglect the problem of unilateral colour blindness! The book contains many valuable references, is illustrated by line diagrams and has been well translated.

DIE LUNGENARTERIENBAHN BEI ANGEBORENEN HERZFEHLERN. Von Kurt Köhn und Marrianne Richter. Stuttgart: Georg Thieme. 1958. Pp. viii + 112. Dm. 29.50. The authors discuss the anatomy and physiology of the pulmonary circulation and review the literature of pathological changes in the pulmonary circulation in some detail. Their own contribution is based on a study of the lungs in forty-one subjects with congenital cardiovascular lesions who came to autopsy. The material is grouped into those with plethora of the pulmonary circulation from left to right shunting of blood, those with diminished pulmonary circulation from stenosis of the right ventricular outflow tract and a small group with aortic and sub-aortic stenoses.

Parietal thromboses appear to be common to both the groups with increased and decreased pulmonary flow and the degenerative changes of intimal proliferation, elastic reduplication and hyaline or fibrous changes of media of the smaller arteries are commonly found with increased pulmonary circulation. The presence of arterio-venous communications is noted. The significance of the vascular changes is discussed.

HIGH ARTERIAL PRESSURE. By F. H. Smirk. Oxford: Blackwell Scientific Publications. 1958. Pp. xxxvi + 764. £3, 15s. Professor Smirk, whose work on the treatment of high blood pressure has attracted wide interest during the past decade, has undertaken the formidable task of writing a comprehensive account of the physiology of blood pressure regulation and the natural history, pathology, diagnosis and treatment of hypertension.

Physiologists will find a well documented discussion of the mechanisms of blood pressure control and of the racial, constitutional, environmental and other factors which modify it. But the book appears to be addressed primarily to the practising clinician and it is to the clinician that it will probably be of most interest. Professor Smirk's numerous original contributions to the therapeutics of hypertension have already placed us in his debt and it is appropriate that we should have his considered views within the compass of a single volume.

URINE AND THE URINARY SEDIMENT. By R. W. Lippmann. Illinois: Charles C. Thomas. 2nd edition. 1957. Pp. ix + 140. £3, 3s. This small monograph gives full details of the methods of examining the urine, which are suitable for a small clinical laboratory. Special emphasis is placed on the microscopic examination and there are ninety-three coloured photographs of urinary sediments. These are beautifully reproduced and provide a valuable reference for the clinician. The book can be recommended as a practical laboratory guide.

NUTRITION AND BEHAVIOUR. Edited by J. Brozek. Proceedings of a Symposium held at the University of Minnesota. New York: The National Vitamin Foundation Inc. 1957. Pp. 124. $2.50. This symposium gives a useful account of various aspects of hunger and appetite with special emphasis on psychological and behavioural aspects. There is a good discussion of the factors that determine satiety and excessive food intake with special reference to the problems of obesity.

ANÆSTHESIA. *Brit. Med. Bull.* **14,** Number 1, January. 1958. London: The British Council. Pp. xiv + 72. £1. In this number physicists in anæsthetic departments consolidate their position and help to make anæsthesia one of the more quantitative branches of medical practice. There are also a number of somewhat dogmatic clinical opinions on the mode of action of drugs which suggest that the process of enlightenment is not yet complete. There are no outstanding advances, but present problems and trends are analyzed fairly and profitably.

SEKRETIONSSTUDIEN AM PANCREAS. By V. Becker. Stuttgart: Georg Thieme. 1957. Pp. vii + 119. D.M. 23.60. This is a short, well-produced monograph dealing with the exocrine secretion of the pancreas. The emphasis is on the histopathological changes associated in man with disturbances in function of the acinar tissue and on the sequence of events that from various initiating causes may lead to its destruction. The book is consequently of greater interest to the pathologist than to the physiologist. The latter may nevertheless find it useful for reference since it contains an adequate and well-written review of the physiology of pancreatic secretion and the conclusions are based on experimental as well as on clinical material.

REFLEXOGENIC AREAS OF THE CARDIOVASCULAR SYSTEM. By C. Heymans and E. Neil. London: J. & A. Churchill, Ltd. 1958. Pp. viii + 271. £2, 16s. This is a careful and critical review of present day knowledge of cardiovascular reflexes and their afferent paths. Although the arterial baroreceptors and their reflexes are faithfully dealt with, and the earlier work on the carotid sinus is reviewed, the book is far from being limited to these receptors. The chemoreceptors and the atrial and pulmonary receptors are discussed in detail and illustrated by many attractive records made by Neil. A vigorous style and downright judgments make this book a refreshing change from the noncommittal enumeration of papers which all too frequently make up topical reviews. The bibliography is very extensive. This is one of the most valuable recent monographs on physiology.

INSTRUMENTAL ANALYSIS. By P. Delahay. New York: The Macmillan Co. 1957. Pp. xi + 384. £2, 15s. 6d. This book will be useful to undergraduate and post-graduate students in the field of analytical chemistry, who are trying to acquire rapidly a wide superficial knowledge of modern physical techniques. Parts of it will be of value to workers in the biological fields, but not to those requiring a deeper understanding of these techniques or their application.

NORMAL VARIATIONS IN TOTAL KETONE BODIES IN SERUM AND URINE OF HEALTHY YOUNG MEN.

By R. E. Johnson, F. Sargent, II and R. Passmore. From the Departments of Physiology, University of Illinois, Urbana, U.S.A. and Edinburgh University.

(*Received for publication 22nd March* 1958)

To establish normal values and ranges for studies of ketosis, total ketone bodies have been estimated in the urine and serum of 208 healthy young men, eating non-ketogenic adequate diets and engaged in moderate daily physical activity. Under standardized post-absorptive conditions timed specimens of urine were collected, and venous blood was drawn. The urinary excretion rate of ketone bodies differed significantly in summer and winter. In hot weather the mean rate was 0·9 μ-mole/min.; in cold weather it was 2·8 μ-mole/min.; in cool weather it was 1·7 μ-mole/min. For serum, regardless of season, the mean was 0·7 m-mole/l. If two standard deviations be taken as the normal range, it is suggested that for most conditions the upper limit of normal for serum be considered as 1·4 m-mole/l. and for urine 5 μ-mole/min.

An interest in ketosis which may arise in healthy men from nutritional causes, especially starvation, and after prolonged exercise (the Courtice-Douglas effect), led us to inquire: "What are the normal values for total ketone bodies in blood and urine?" A systematic study of changes in ketone metabolism in man demands knowledge of the normal, yet present information is scanty and unsatisfactory. For instance, the *Handbook of Biological Data* [Albritton, 1953] has no separate lists for the individual ketone bodies in urine, and only gives the total excretion in daily specimens collected at random without regard to previous dietary history or activity of the subjects. Furthermore, analytical methods have been unsatisfactory and tedious. For beta-hydroxybutyric acid, which may account for half or more of the total ketone bodies, no qualitative test exists, and hitherto many quantitative methods have not given true values for it.

During metabolic studies with 208 subjects, we have been able to collect specimens of blood and urine under standardized post-absorptive conditions and to analyze these with satisfactory quantitative and qualitative methods. Knowledge of the subjects' previous dietary history, daily activity, and environment was adequate. Hence, we are able to assert that for healthy young men we know the normal range of concentration of total ketone bodies in serum; the normal rate of excretion of these substances in urine under post-absorptive conditions; and the relation between quantitative and qualitative examination of urine collected under these conditions. Qualitative results have been reported by Sargent and Consolazio [1950] which support the view that exposure to cold increases the rate of excretion of acetone plus acetoacetic acid. We have confirmed this observation quantitatively for total ketone bodies.

METHODS

Subjects.—Three studies have been conducted: summer, 1955 (Indiana); winter, 1954 (Wisconsin); and winter, 1957 (Edinburgh). In each of the first two, ninety-nine healthy young airmen were the subjects, and in the last, ten healthy young medical students.

Activity and Environment.—Daily work was moderate, consisting of walking outdoors to and from classes, other duties and meals. At most other times the subjects were living a sedentary life and were not much exposed to the weather, even though the outdoor temperatures were rather severe.

TABLE I.—SUBJECTS AND REGIMENS

Groups of subjects	Subjects number	Duration of regimen days	Total Calories Cal/day	Percentage of Calories from		
				Protein	Fat	Carbohydrate
		Summer, 1955, Indiana				
A *	88	14	3400	15	34	51
B †	11	14	4100	14	41	45
		Winter, 1954, Wisconsin				
C *	87	14	3200	14	33	53
D †	12	14	4500	15	40	45
		Winter, 1957, Edinburgh				
E ‡	10	2	3000	15	45	40

* This diet consisted of canned items in large variety with fresh bread.

† This diet consisted of abundant amounts of fresh and frozen foods in wide variety.

‡ This diet consisted of dried milk solids with hydrogenated vegetable fat and carbohydrate added.

Diet.—This is described summarily in Table I. The subjects were under continuous nutritional observation. It was intended that the amounts of the proximate principles of their diets should be representative of usual North American and British practice and should be adequate in quality. Unlimited fluids were provided. In the two American experiments the daily physical activity of all subjects was the same. Groups B and D received a luxury diet of the best procurable natural foods.

Collection of Urine and Blood.—On the night before the observation food was last eaten at about 8 P.M. In the morning 100–200 ml. of water was drunk and the subjects came, without food, to the laboratory. In the 1954 and 1955 experiments the subjects emptied their bladders and then lay down for 2 hr. without smoking, drinking or eating. During this period a specimen of urine, timed to the nearest minute, was collected and its volume measured to the nearest ml. Venous blood was drawn at the midpoint. In the 1957 experiments the subjects were ambulatory and no blood was drawn.

Quantitative Analysis.—There is as yet no rapid and accurate quantitative method for determining total ketone bodies. In particular, the oxidation of beta-hydroxybutyric acid to acetoacetic acid by dichromate at 100° C. has usually been reported as only 60–80 per cent complete. We have succeeded in making this yield 100 per cent by using temperatures over 100° C. together with metaphosphoric acid, which catalyzes the oxidation. This substance has the further advantage of being an excellent protein precipitant.

For total ketone bodies, 10 ml. of 0·09 M-metaphosphoric acid are placed in a cone-tipped centrifuge tube. To this is added 0·5 ml. of serum or urine, and the whole is mixed by inversion. After centrifuging, 3 ml. of the supernatant are placed in an ampoule (or, rather better, in a screw-topped tube fitted with a teflon plug) and then 1 ml. of 3 N-sulphuric acid containing potassium dichromate in 0·02 M concentration is added. The ampoule or tube is sealed and autoclaved for 1 hr. at 15 lb. pressure. After cooling, the mixture is analyzed by the method of Michaels, Morgan, Liebert and Kinsell [1951] or by the method of Thin and Robertson [1952]. The first relies on the formation of the 2 : 4 dinitrophenylhydrazone of acetone, which is extracted into carbon tetrachloride; in the second acetone diffuses in a Conway plate into alkaline salicylaldehyde. In both methods the intensity of the final colour is measured. Reagent blanks and standards of calcium zinc beta-hydroxybutyrate are included in each batch of samples.

Qualitative Analysis.—The method of Rothera [1908] has been standardized to give maximum sensitivity and uniform comparability from time to time. About 5 g. of anhydrous ammonium sulphate is added to a clean dry test tube; there must be enough to saturate the final solution and leave an undissolved excess. In succession and with mixing after successive additions, 0·5 ml. of urine, 0·2 ml. of freshly prepared 0·07 M-sodium nitroprusside, and 0·2 ml. of 5-N-ammonium hydroxide are added. Colour is recorded as 0–4 plus at $\frac{1}{2}$, 5 and 10 min., and the maximum reading is accepted.

RESULTS

Quantitative: *Serum.*—Between winter and summer there were no significant differences in concentrations of total ketone bodies in the serum (Table II), and no significant differences between groups A and C and groups B and D. Therefore, a weighted mean for all subjects is justified. For all 198 specimens the mean was 0·71 m-mole/l., the standard deviation ±0·21 m-mole/l. and the coefficient of variation 30 per cent.

TABLE II.—CONCENTRATIONS OF TOTAL KETONE BODIES IN SERUM

Group of subjects	Observations number	Mean m-mole/l.	Standard deviation m-mole/l.	Coefficient of variation per cent	Outdoor temperature ° C.
		Summer, 1955, Indiana			
A	88	0·64	0·20	31	+26 to +27
B	11	0·68	0·17	25	+26 to +27
		Winter, 1954, Wisconsin			
C	87	0·75	0·19	25	−7 to +7
D	12	0·96	0·38	39	−7 to +7
		Weighted means, Indiana plus Wisconsin			
AC	175	0·69	0·20	29	. .
BD	23	0·83	0·28	34	. .
all together	198	0·71	0·21	30	. .

Statistical analysis: by students' "t" test, no differences between groups or seasons were significant at a probability level of 5 per cent.

Quantitative: Urine.—The urinary excretion of total ketone bodies varied significantly with season (Table III). In severe winter cold excretion was greatest; in warm summer weather, least; and in the moderate winter weather of Scotland, intermediate. In all groups, the coefficient of variation was larger than for serum. No weighted means would be statistically permissible for urine.

TABLE III.—URINARY EXCRETION OF TOTAL KETONE BODIES

Group of subjects	Observations number	Mean μ-mole/min.	Standard deviation μ-mole/min.	Coefficient of variation per cent	Outdoor temperature ° C.
		Summer, 1955, Indiana			
A	88	0·80	0·30	38	+26 to +27
B	11	1·08	0·85	79	+26 to +27
		Winter, 1954, Wisconsin			
C	87	2·56	1·28	50	−7 to +7
D	12	3·10	1·32	43	−7 to +7
		Winter, 1957, Edinburgh			
E	20	1·73	0·94	54	+8 to +14

Statistical analysis: by students' "t" test, differences were not significant between groups within summer and within winter at the 5 per cent level. All differences between A and B groups and C and D groups were significant at the 1 per cent level or below. Differences between the E groups and all AB and CD groups were significant at the 5 per cent level or below.

TABLE IV.—ROTHERA REACTION IN URINE

Groups	Observations number	Strength of reaction					
		0	tr	1+	2+	3+	4+
		Summer, 1955, Indiana					
A	170	169	1	..	..	..	..
B	24	24	..	..	..	..	..
		Winter, 1954, Wisconsin					
C	158	116	18	15	4	3	2
D	22	13	3	3	2	..	1
		Winter, 1957, Edinburgh					
E	20	19	1	..	..	..	..

Statistical analysis: by the "Chi Square" test, there was no difference between A and C or B and D groups within summer or within winter, significant at the 5 per cent level. There was a difference between both winter 1954 groups, and all summer 1955 and winter 1957 groups, significant at the 0·01 per cent level.

Qualitative: Urine.—There was a significant difference between severe winter weather and summer or mild winter weather (Table IV). Among the 394 specimens tested, positive reactions of +1 or above were obtained only in Wisconsin. These qualitative findings for acetone plus acetoacetic acid are in agreement with the quantitative results of Table III for total ketone bodies; and they confirm the observations of Sargent and Consolazio [1950] for conditions of extreme cold.

Discussion

The interpretation of means and ranges is always a matter of statistical judgment. For total ketone bodies in serum, we suggest that twice the standard deviation be considered the normal range. Our groups B and D had the higher mean figure. If this is taken and two standard deviations added, the upper limit of normal for total ketone bodies in serum is 1·4 m-mole/l., regardless of season. The existence of a correlation with temperature complicates the statistics for urinary excretion. However, as a general rule we should place 5 μ-mole/min. as the upper limit of normal, realizing that it will be less in warm weather. Recalculation of data in the *Handbook of Biological Data* [Table 116, Albritton, 1953] gave an average of 2·3 μ-mole/min., and an upper limit of 5·7, for daily specimens collected without control of diet or environment.

For physiological studies of ketone metabolism we urge the use of units of chemical equivalence, reform of inaccurate terminology, and standardization of conditions for collecting urine and blood.

It has been common for investigators to express their results in terms of mg. of acetone per 100 ml. of urine or blood. This can lead to confused thinking. Table V demonstrates that, if one considers only concentration by weight in the blood, glucose would appear to be the predominant carbohydrate (or derivative), and total fatty acid the predominant fat (or derivative). As judged by equivalent chemical units, however, such is certainly not always the case. In nutritional ketosis the molar concentration of total ketone bodies may equal that of glucose and far exceed that of free fatty acids; and after anærobic work of short duration, the molar concentration of lactic acid is often at least double that of glucose. Even metabolites that are normally present in very small concentrations, like pyruvic acid, under some circumstances may become quantitatively important.

Table V.—Some Illustrative Concentrations of Intermediary Metabolites in Blood

Metabolite	Conditions	By weight mg./100 ml.	By chemical equivalence m-mole/l.
Glucose . . .	At rest	81	4·5
Free fatty acid . .	At rest	28	1·0
Lactic acid . .	At rest	9	1·0
Lactic acid . .	After exercise	81	9·0
Pyruvic acid . .	At rest	2	0·23
Pyruvic acid . .	In severe beriberi	10	1·1
Ketone bodies . .	At rest	5	0·8
	In nutritional ketosis	23	4·0

Terminology for discussions of ketosis needs modification. The terms "ketonuria" and "ketonæmia" were originally given a pathological significance, because they were based on qualitative tests which could not detect small concentrations. However, modern quantitative methods show that there are always measurable concentrations of ketone bodies in urine and blood. Therefore, the pathological connotation might better be served by the terms "hyperketonuria" and "hyperketonæmia".

We recommend strongly the routine use of timed specimens of urine collected when the subjects are post-absorptive, and blood drawn during the same period. The two advantages that would accrue would be comparability of data from laboratory to laboratory, and applicability of data to calculation of renal parameters. In view of the correlation between temperature and renal excretion of total ketone bodies, we recommend control of the environment, or, if that is not possible, recording of environmental conditions under which the study may be conducted.

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In 1957-1958 R. E. Johnson was on sabbatical leave and the recipient of a U.S. National Science Foundation Senior Post-doctoral Research Fellowship for study at Edinburgh University. F. Sargent, II, was on sabbatical leave and the recipient of a John Simon Guggenheim Memorial Foundation Fellowship for study at Oxford University.

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THE EFFECTS OF ENVIRONMENT AND OTHER FACTORS ON NUTRITIONAL KETOSIS.

By F. Sargent, II, R. E. Johnson, Evelyn Robbins and Laura Sawyer. From the Department of Physiology, University of Illinois, Urbana, U.S.A.

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During studies of survival rations for air crews, nutritional observations were made on 198 healthy young men continuously for 6 weeks. In forty-eight of these subjects nutritional ketosis was produced by 2 weeks of complete starvation; by a diet high in fat, but low in protein and carbohydrates; or by a diet high in fat and protein, but devoid of carbohydrate. Paired feeding techniques were used for groups of two to four subjects. By factorial analysis the relation was determined between nutritional ketosis and five variables: (1) environmental temperature (winter and summer); (2) total caloric intake (0, 1000 and 2000 Cal/day); (3) water balance (unlimited water and water restricted to 910 ml./day); (4) duration of regimen (1 and 2 weeks); and (5) work load (a daily march of 5 and 20 km.). As judged by ketonuria and ketonæmia, the ketogenicity of any diet (including starvation) was reduced significantly by increase in environmental temperature, by increase of caloric intake with the same diet, and by a reduction of fluid intake. Ketosis diminished in the second week of any regimen, and tended to be less with increased daily work. These results may modify orthodox concepts of nutritional ketosis, based on the "ketogenic/antiketogenic ratio" of Shaffer.

Shaffer in 1921 postulated that a "ketogenic/antiketogenic ratio" in the metabolic mixture determined whether or not oxidation of fat to CO_2 and water would be complete and, if incomplete, the magnitude of the ensuing ketosis. This general concept, although modified with time, is still widely used. Thus Brody [1945] wrote "The current idea seems to be that carbohydrates are not antiketogenic in Shaffer's coupled reaction sense but, more simply, in the sense that when the carbohydrate oxidation is low, fat oxidation is correspondingly high, and high fat metabolism necessarily involves relatively high ketone formation." Deuel [1957] recognized several factors which diminish carbohydrate oxidation, increase fat metabolism, or both. These include starvation, either total or specific carbohydrate deprivation; hypoinsulinism; fat mobilizing substances from the pituitary; other neuro-endocrine factors; species; age; sex; "adaptation"; nutritional state; and "many other factors" which Deuel does not specify. Clearly nutritional ketosis is not simple.

A study of air crews living on various survival rations provided an opportunity to investigate the effect of some of these factors on nutritional ketosis.

Methods

Subjects and Environment.—Two separate experiments were conducted with different subjects, one in the winter of 1953–1954 in Wisconsin, the other in the summer of 1955 in Indiana. Experimental designs were identical. After a period of 2 weeks, when all subjects lived on adequate diets with unlimited water, the men lived for 2 weeks in bivouac under conditions similar to those which survivors from a crash or forced landing might expect to find. They had adequate tents and clothing for the season, but they were exposed all the time to the stresses of the environment, cold down to −21° C. in 1954, and moist heat up to +41° C. in 1955. Numbers of subjects and outdoor temperatures on the day of the weekly examination are given in Table I, together with information on daily work load and water intake. Some simulated active escape and evasion with a daily march of 20 km., others

TABLE I.—SUBJECTS, WORK LOAD, WATER INTAKE AND ENVIRONMENTAL TEMPERATURE

Identification of group	Subjects at start number	Daily work km. march	Water intake l./day	Mean temperature day of examination	
				Week 1 ° C.	Week 2 ° C.
		Winter, 1954			
Hard work U . . .	21	20	No limit	−7	+2
Hard work L . . .	22	20	0·91	−7	+2
Light work U . . .	22	5	No limit	−7	+2
Light work L . . .	22	5	0·91	−7	+2
Controls	12	Variable	No limit	−7	+2
Total	99				
		Summer, 1955			
Hard work U . . .	22	20	No limit	+28	+26
Hard work L . . .	22	20	0·91	+28	+26
Light work U . . .	22	5	No limit	+27	+26
Light work L . . .	22	5	0·91	+27	+26
Controls	11	Variable	No limit	+27–28	+26
Total	99				

TABLE II.—COMPOSITION OF DIETS

General composition of diet	Total calories Cal/day	Percentage distribution of calories			Code in tables
		Protein	Fat	CHO	
Starvation	0	..	..	..	STO
Spice drops, starch-jelly, sugar candy	1000	0	0	100	0/100/0 1000
	2000	0	0	100	0/100/0 2000
Saltine biscuits, oleomargarine .	1000	2	78	20	2/20/78 1000
	2000	2	78	20	2/20/78 2000
Meat bar (pemmican) . .	1000	30	70	0	30/0/70 1000
	2000	30	70	0	30/0/70 2000
"Normal mixture," meat bar, biscuits, jam, raisins, catsup .	1000	15	33	52	15/52/33 1000
	2000	15	33	52	15/52/33 2000
	3000	15	33	52	15/52/33 3000 or N 3000
"Control"; fresh and frozen foods in variety . . .	3700	14	41	45	FRA, Control

"sat by the aircraft" with a daily march of only 5 km. Some had unlimited water to drink, others were restricted to 910 ml./day. The Table includes all 198 subjects who started the 2 weeks, but eleven were withdrawn before the end for medical reasons.

Diets.—These are summarized in Table II. The three non-ketogenic diets were pure carbohydrate, "normal mixture", and the control diet. The three ketogenic diets were starvation; biscuits and oleomargarine; and meat bar. They were all taken daily in the prescribed amounts throughout the two weeks.

Specimens and Analyses.—Post-absorptive specimens of urine and blood were collected at the end of each week, and analyzed for total ketone bodies. These methods are described in detail in a previous paper [Johnson, Sargent and Passmore, 1958].

RESULTS

Table III gives all the data for urine and Table IV all for serum. These Tables are too complex for easy inspection. Therefore, we shall discuss first the three ketogenic diets, and then the non-ketogenic diets.

TABLE III.—RATE OF EXCRETION OF TOTAL KETONE BODIES IN URINE (μ-MOLE/MIN.)

(Each figure is the mean for two or more subjects)

Regimen *		Summer, 1955, Indiana				Winter, 1954, Wisconsin			
		Hard work		Light work		Hard work		Light work	
		Week 1	Week 2	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
STO	U	2·8	4·8	15·7	2·5	19·3	1·4	44·9	6·7
	L	2·8	3·4	28·9	11·3	12·1	5·6	34·4	10·1
0/100/0	U	0·3	1·9	0·7	0·5	3·0	0·6	3·3	1·3
1000	L	0·8	0·8	1·4	0·5	3·2	2·7	2·9	1·2
0/100/0	U	0·3	1·2	0·7	0·6	1·9	1·0	2·7	1·9
2000	L	0·5	0·7	0·8	0·2	0·7	0·6	2·0	2·2
2/20/78	U	4·0	4·2	4·4	1·1	18·1	6·0	25·2	22·3
1000	L	2·1	1·0	16·4	5·3	6·2	1·8	21·7	15·3
2/20/78	U	2·7	2·8	3·4	1·5	8·4	6·2	13·3	31·3
2000	L	1·1	0·8	5·9	0·8	8·4	3·4	5·3	5·1
15/52/33	U	1·7	2·9	0·7	0·8	3·4	1·6	3·3	1·9
1000	L	0·8	. .	1·5	1·5	3·4	1·8	2·8	3·3
15/52/33	U	0·5	1·5	1·7	2·0	4·4	3·1	6·0	3·0
2000	L	1·6	0·8	0·6	0·5	4·5	1·7	4·1	2·2
15/52/33	U	0·7	1·6	0·7	0·9	2·9	2·6	3·8	2·8
3000	L	0·7	0·6	. .	. .	4·6	2·6	3·0	2·6
30/0/70	U	4·9	10·1	6·2	2·2	52·4	15·6	21·9	21·8
1000	L	1·6	3·9	14·8	4·0	15·7	6·0	6·3	3·6
30/0/70	U	4·8	6·9	2·4	1·3	32·2	9·8	13·2	6·9
2000	L	1·2	2·9	1·4	0·6	10·8	3·8	8·4	5·3
Control	U	0·6	0·9	0·9	0·9	3·3	2·8	2·9	3·4

* The three ketogenic diets were starvation (STO) and two that were high in fat (2/20/78 and 30/0/70). See Table II for details.

Ketogenic Diets.—In figs. 1–5 each point compares the rates of excretion of total ketone bodies for two pair-fed groups of subjects; for each pair ketogenic regimens were similar, except for the season (fig. 1), calorie intake (fig. 2), water intake (fig. 3), duration of regimen (fig. 4) or daily work load (fig. 5). Only one variable is assigned to each chart. An oblique line has been drawn in each figure on which the points would be expected to fall if the factor concerned was unassociated with ketosis. The original data have been subjected to "Chi Square" analysis the results of which are given for urine in the legends to the figures, and for serum at the bottom of Table IV.

TABLE IV.—CONCENTRATION OF TOTAL KETONE BODIES IN SERUM (M-MOLE/L.)

Each figure is the mean for two or more subjects)

		Summer, 1955, Indiana				Winter, 1954, Wisconsin			
Regimen *		Hard work		Light work		Hard work		Light work	
		Week 1	Week 2	Week 1	Week 2	Week 1	Week 2	Week 1	Week 2
STO	U	1·6	1·6	1·9	4·3	4·7	4·2	4·8	4·3
	L	4·2	1·9	2·5	5·0	4·0	2·5	4·6	1·8
0/100/0	U	0·5	0·5	0·9	0·7	1·6	0·7	0·8	0·7
1000	L	0·7	0·4	0·3	0·6	1·5	0·8	0·9	0·9
0/100/0	U	0·4	0·5	0·6	0·4	0·8	0·5	0·6	0·5
2000	L	0·4	0·5	0·2	0·4	1·0	0·5	0·5	0·5
2/20/78	U	0·8	2·6	1·1	1·2	3·2	3·0	4·0	3·3
1000	L	2·3	0·7	1·0	2·1	2·7	1·7	4·1	3·9
2/20/78	U	0·6	0·8	0·8	0·5	2·7	1·7	2·1	1·8
2000	L	2·2	0·6	0·7	0·6	1·9	1·6	1·8	1·2
15/52/33	U	0·7	0·7	0·7	0·7	1·2	1·1	1·2	0·6
1000	L	0·8	..	0·7	0·7	1·2	0·8	1·2	1·4
15/52/33	U	0·4	0·7	0·6	0·5	1·3	1·1	1·1	1·0
2000	L	0·7	0·7	0·7	0·8	1·1	0·7	0·9	0·7
15/52/33	U	0·5	0·7	0·4	0·6	1·1	1·6	0·9	0·6
3000	L	0·4	0·5	..	..	0·6	0·5	0·6	0·6
30/0/70	U	0·9	2·6	2·5	1·5	6·6	5·2	3·7	4·6
1000	L	3·2	0·9	1·4	1·0	3·4	2·7	2·5	2·3
30/0/70	U	0·8	2·0	0·7	0·6	3·7	3·6	2·6	2·5
2000	L	1·9	1·1	0·5	0·8	3·0	1·8	2·4	1·9
Control	U	0·5	0·7	0·6	0·8	0·7	0·6	1·0	0·8

* The three ketogenic diets were starvation (STO) and two that were high in fat (2/20/78 and 30/0/70). See Table II for details. By the "Chi Square" test, the following probabilities were calculated: summer > winter, 0·001 per cent; U > L, 3 per cent; 1000 > 2000, 0·001 per cent; week 1 > week 2, 1 per cent; light work > hard work, 50 per cent. Data were not rounded to the nearest tenth.

Ketonuria was less in summer than in winter (fig. 1), as was ketonæmia. Doubling the caloric intake of the same diet diminished ketonuria (fig. 2) and ketonæmia. Restriction of water intake caused ketonuria to be smaller (fig. 3) as well as ketonæmia. Ketosis was less in the second than in the first week (fig. 4). The results for daily work were only suggestive. In the groups doing light work ketonuria was less than in the hard working groups (fig. 5), but only at a probability of 10 per cent; serum concentrations were not even suggestively different (Table IV).

Non-Ketogenic Diets.—The results with these diets are set out in Tables III and IV, but have not been charted. All the values are below the upper

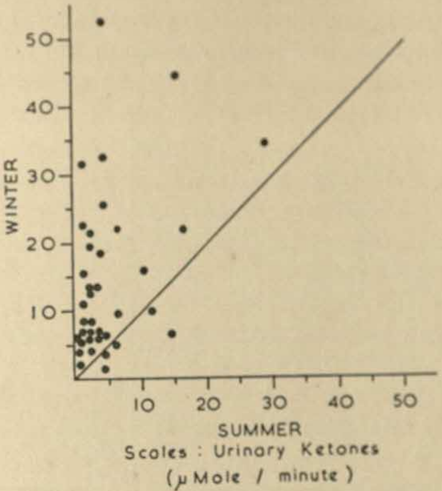

FIG. 1.—Urinary excretion of total ketone bodies for three ketogenic regimens in summer and winter. Both scales, μ-mole/min. The X^2 probability for summer < winter is ·001 per cent.

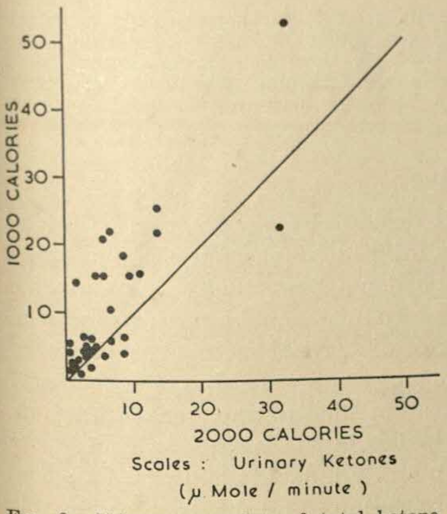

FIG. 2.—Urinary excretion of total ketone bodies at 1000 and 2000 Cal/day of the same ketogenic diets. Both scales, μ-mole/min. The X^2 probability for 1000 Cal > 2000 Cal is ·001 per cent.

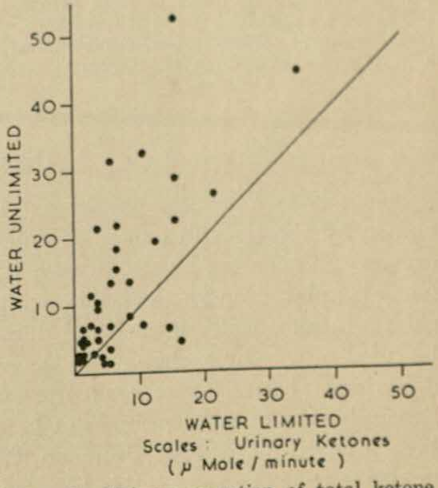

FIG. 3.—Urinary excretion of total ketone bodies for three ketogenic regimens when water intake was limited or unlimited. Both scales μ-mole/min. The X^2 probability for unlimited > limited is 3 per cent.

limit of normal as defined by Johnson *et al.* [1958], except the urinary excretion of one pair of subjects, and the serum concentration of two pairs. These exceptions occurred in the winter in the first week. However, even although the absolute values for ketonæmia were low, the same relative differences were found as in the case of the ketogenic diets. Higher serum concentrations were associated with winter, with lower calorie intake, with unlimited water, and with the first week of the diet, at a 5 per cent level of probability. For ketonuria, only the increase in winter was significant; the other differences were only suggestive.

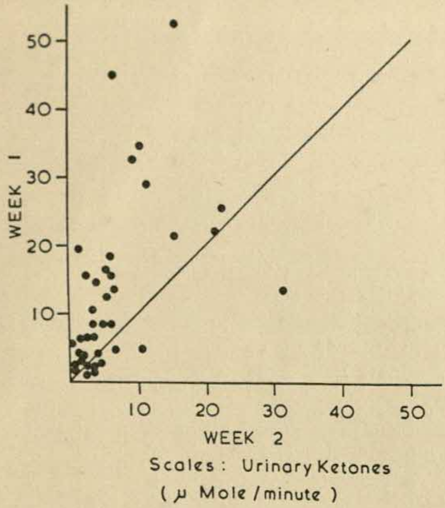

Fig. 4.—Urinary excretion of total ketone bodies for 1 week and 2 weeks of three ketogenic regimens. Both scales, μ-mole/min. The X^2 probability for week 1 > week 2 is 1 per cent.

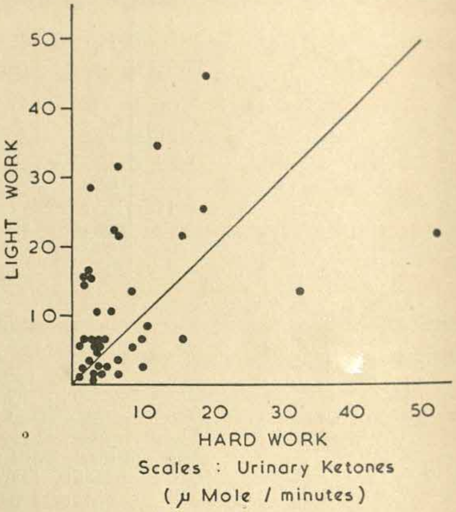

Fig. 5.—Urinary excretion of total ketone bodies during hard daily work and light daily work on three ketogenic regimens. Both scales, μ-mole/min. The X^2 probability for light work > hard work is above 10 per cent.

Discussion

Ketosis is generally considered to be caused by production of ketone bodies by the liver in excess of amounts utilized by the muscles and other organs and excreted by the kidneys [Campbell and Best, 1956]. As a physiological explanation for the relationship between nutritional ketosis and season, caloric intake, water intake and duration of diet, renal factors can probably be ruled out. First, changes in the serum and urine were always in the same direction; hence there was no evidence of fluctuating renal function. Secondly, the renal clearances of ketone bodies by these same subjects have been studied by Sargent, Johnson, Wogan and Pandazi [1958] who found no evidence that the very active tubular reabsorption of total ketone bodies was significantly altered in relation to any of the four variables. If peripheral utilization were an important factor, hard work might be expected to diminish ketonuria. As was shown in fig. 5 differences were only suggestive, not significant. By exclusion, therefore, we must consider changes in hepatic production to be responsible for the variations of the level of ketone bodies in serum and urine. It is fairly well established that hepatic ketogenesis is under a neuroendocrine control, in which insulin inhibits the action of fat mobilizing factors of the pituitary [Campbell and Best, 1956], produced most actively in starvation or absolute carbohydrate deprivation. It could be that exposure to cold stimulates the production of a fat mobilizing factor, which would cause ketonæmia and ketonuria to be greater in winter than in summer. Increase of total calories of the actual ketogenic diets we used would increase the absolute intake of carbohydrate or of protein. Hence one might expect ketonæmia and ketonuria to be less at 2000 than at 1000 Cal/day, even though the "ketogenic/antiketogenic ratio" of the diet was constant. Other authors [cited by Deuel, 1957] have speculated that prolonged subsistence on a ketogenic diet leads the liver to produce "adaptive enzymes", with diminution of ketogenesis, all other factors remaining constant. About the effect of dehydration, we cannot at this time even speculate fruitfully.

No matter what the physiological explanation of the changes, it seems clear not only that the magnitude of any dietary ketosis may be markedly altered by temperature, by calorie intake, by water intake and by the duration of the regimen. Also these variables are related to alterations of ketone body metabolism even in subjects on a non-ketogenic diet. The phenomenon of nutritional ketosis is more complex than has yet been appreciated; the "ketogenic/antiketogenic ratio" of Shaffer [1921] is only one factor, important to be sure, but not fixed in its effects.

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In 1957–1958 R. E. Johnson and F. Sargent, II, were on sabbatical leave from the University of Illinois. The former was a U.S. National Science Foundation Senior Post-doctoral Research Fellow at Edinburgh University. The latter was a John Simon Guggenheim Memorial Foundation Fellow at Oxford University.

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THE MODIFICATION OF POST-EXERCISE KETOSIS (THE COURTICE-DOUGLAS EFFECT) BY ENVIRONMENTAL TEMPERATURE AND WATER BALANCE.

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The ketosis that may arise following a 10-mile walk in the post-absorptive state (the Courtice-Douglas effect) has been studied in ten young men. Each completed two walks followed by 6 hr. of recovery. One experiment was in a warm environment (about 24° C.), the other in a cool environment (about 12° C.). During both measured amounts of water were given, sufficient to maintain a good flow of urine. For the previous 2 days an artificial diet was eaten, providing 3000 Cal/day (15 per cent from protein, 45 per cent from fat and 40 per cent from carbohydrate) and unlimited fluids. In all experiments, rates of excretion of total ketone bodies increased after the walk. If ketosis is defined as a ketonæmia above 1·4 m-mole/l. of serum or a ketonuria above 5 μ-mole/min., then it developed in nine of the twenty experiments, six being in the cool environment. The greatest ketosis was observed when water balance was positive. It did not develop when there was a large negative water balance. The Courtice-Douglas effect appears to be a general metabolic phenomenon, whose magnitude may be modified by environmental temperature and by hydration, as well as by previous diet.

Introduction

Ketosis often develops during rest after prolonged moderate exercise in a post-absorptive state [Courtice and Douglas, 1936]. This may be of importance to a general understanding of the metabolism of ketone bodies in man. Yet the Courtice-Douglas effect has received but scant attention. For example the phenomenon is not even mentioned by Campbell and Best [1956] or by Deuel [1957] in long reviews on the physiology and biochemistry of ketosis. The findings of Sargent, Johnson, Robbins and Sawyer [1958] that the magnitude of a nutritional ketosis may be affected, sometimes markedly, (1) by the environmental temperature, (2) by the total calories of a ketogenic diet, (3) by the water intake and (4) by the duration of the regimen, have led us to study the Courtice-Douglas effect in ten young men at two different environmental temperatures, one cool, the other warm. Their diet was adequate and controlled for two days, and their water intake during and after the exercise was controlled but liberal.

Methods

The Subjects.—These were all healthy young male medical students, whose physical characteristics were in no way unusual (Table I). Cas. and Gra. were members of university rugby teams and in full training. Bar. was a regular player of squash. The other seven were not regular participants in any sport.

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Diet.—During the 48 hr. before the experiment each subject ate a diet providing 3000 Cal. daily. A mixture was made from dried milk solids (Complan), hydrogenated vegetable oil (Prosparol) and glucose, which provided 450 Cal. from protein, 1350 from fat and 1200 from carbohydrate. This provided 60 m.equiv. of sodium, 69 of chloride and 90 of potassium daily. Water intake was unrestricted. On the day of the experiment the subject arrived at the laboratory without food. During the 8½ hr. of the experiment he received no food, but at regular intervals drank measured amounts of water calculated to maintain a positive water balance.

Environment, Exercise and Recovery.—The subject walked for 2½ hr. at 6·7 km./hr. (4 m.p.h.). For the warm experiment he walked on a treadmill in a room maintained at 24° C. For the cool experiment he walked out of doors on a flat circular track. Temperatures varied from 8° to 14° C. After the walk he lay down for 6 hr. at a temperature approximately the same as that in which he had walked. No smoking was permitted, and the subject arose only every 2 hr. to urinate. As part of the statistical design half the subjects walked first in the warm and half first in the cool.

TABLE I.—SUBJECTS

Identification	Age Years	Weight kg.	Height cm.
Bar.	21	66·2	175
Cas.	21	66·8	178
Don.	20	58·7	178
Dow.	20	62·9	174
Gra.	21	68·8	175
Dav.	22	52·3	178
Gow.	21	57·6	174
McK.	22	52·0	165
Ash.	22	66·2	173
Bow.	22	61·4	180
Mean	21	61·3	175

Specimens and Analyses.—Timed specimens of urine were collected just before and at the end of the walk, and every 2 hr. during rest. Venous blood was drawn at the fifth hour of rest. Total ketone bodies were estimated as described by Johnson, Sargent and Passmore [1958]. Urinary nitrogen was estimated by a standard Kjeldahl procedure; a copper-selenium catalyst was used during the digestion.

Each half hour during the walk, expired air was measured for 5 min. with a Kofranyi-Michaelis respirator: an aliquot sample was analyzed with the Haldane apparatus. Every hour during the 6 hr. of rest, respiratory measurements were made as during the walk (cool experiments) or with a Douglas bag (warm experiments).

Calculation of the Metabolic Mixture.—The amount of protein, fat and carbohydrate utilized during the walk and the recovery period and the metabolic water were calculated from the amounts of oxygen consumed, carbon dioxide produced and nitrogen excreted in the urine. The factors of Magnus-Levy [1907] were used.

Determination of the Water Balance.—The water ingested was measured and the metabolic water calculated. The weight of water in the urine was obtained by subtracting from the weight of urine the weight of total solids calculated by the Trapp formula [1850]:

$$\text{urinary total solids per litre} = (1000 \times \text{specific gravity} - 1000) \times 2.$$

No fæces were passed in any experiment. The evaporative water loss was obtained by correcting the invisible weight loss for the CO_2 produced and O_2 consumed. The invisible weight loss was determined by weighings of each subject on a balance sensitive to 2 g. at the beginning and end of each walk and at the end of the recovery period. The water balance then equals ingested and metabolic water minus urinary and evaporative water.

RESULTS

Tables II, III and IV give the analyses of ketone bodies in urine and blood, the water balance during the 8½ hr. of the walk and recovery, and the metabolic mixtures for both walk and recovery.

TABLE II.—KETONURIA AND KETONÆMIA

Subject	Total ketone bodies in urine µ-mole/min.						Total ketone bodies in serum m-mole/l.
	Pre	Walk	Hours resting				
			0–2	2–4	4–6	0–6	Fifth hour rest
			A.—Warm environment (21–25° C.)				
Bar.	3·5	6·4	6·3	7·0	7·3	6·9	0·93
Cas.	1·6	3·7	7·9	6·9	8·6	8·0	1·30
Don.	1·7	2·0	2·8	3·1	3·4	3·1	0·52
Dow.	1·5	1·4	2·8	2·8	2·8	2·8	0·55
Gra.	2·3	2·7	3·1	4·2	4·3	3·9	0·72
Dav.	1·0	1·6	1·9	1·6	2·2	2·1	0·32
Gow.	2·2	2·1	2·4	2·7	1·1	2·1	0·36
McK.	1·1	2·6	3·8	5·0	8·5	5·8	2·11
Ash.	0·5	1·4	1·9	1·7	2·0	1·9	0·31
Bow.	0·8	1·4	2·8	2·9	3·0	2·9	0·71
Mean	1·6	2·5	3·6	3·8	4·3	3·7	0·78
			B.—Cool environment (8–14° C.)				
Bar.	2·0	6·3	14·4	10·0	17·3	13·8	1·41
Cas.	2·7	3·6	7·4	8·0	13·8	9·8	1·08
Don.	0·8	3·4	2·4	3·7	5·9	4·0	0·75
Dow.	1·8	2·1	1·9	2·4	0·9	1·8	0·34
Gra.	1·3	1·2	2·6	3·2	2·2	2·6	0·62
Dav.	1·6	1·8	8·0	33·5	44·9	27·8	3·64
Gow.	4·0	2·0	2·6	3·0	4·5	3·3	0·53
McK.	1·2	2·2	3·3	4·4	5·6	4·4	1·54
Ash.	0·7	1·8	2·1	2·6	3·6	2·8	0·96
Bow.	2·6	1·4	6·6	2·9	7·2	5·5	1·22
Mean	1·8	2·6	5·1	7·4	10·6	7·6	1·21

Concordance of Serum Levels and Urinary Rates of Excretion for Ketone Bodies.—Fig. 1 sets out the relevant data from Table II. The serum levels and urinary outputs are clearly related. Subsequently we refer only to the urinary ketones; the same conclusions can be drawn from both, but the urinary data covered the whole experimental period.

The Courtice-Douglas Effect as a General Phenomenon.—Table II shows that in all twenty experiments rates of excretion of ketone bodies at one time during recovery rose above the rate during the walk. In eighteen out of twenty experiments mean rates during recovery were higher than during the walk (fig. 2). These differences existed even at very low absolute rates.

Ketosis and Environmental Temperature.—Fig. 3 shows that in seven out of ten paired experiments ketonuria was greater in the cool than in the warm environment. Johnson *et al.* [1958] have defined ketosis as ketonuria above 5 μ-mole/min. or serum concentration above 1·4 m-mole/l. Ketosis, so defined, occurred at some period in recovery in three of the warm experiments, and in six of the cool experiments (Table II).

TABLE III.—WATER EXCHANGES IN 2½ HR. WALKING PLUS 6 HR. RECOVERY

	Intake		Output		
Subject	Fluid water g.	Metabolic water g.	Water in urine * g.	Evaporative water g.	Balance g.
		A.—Warm environment (21–25° C.)			
Bar.	1800	172	703	1227	+ 42
Cas.	1900	199	979	1473	−353
Don.	1800	168	1584	828	−444
Dow.	1800	195	564	1280	−501
Gra.	1600	215	776	1373	−334
Dav.	1600	149	941	717	−417
Gow.	1700	163	1565	878	−580
McK.	1700	202	1004	1150	−252
Ash.	1700	197	1506	910	−519
Bow.	1700	184	1167	1020	−303
Mean	1730	184	1079	1086	−366
		B.—Cool environment (8–14° C.)			
Bar.	800	177	296	658	+ 23
Cas.	800	147	556	594	−203
Don.	800	146	995	370	−419
Dow.	1000	185	1156	832	−803
Gra.	900	163	1232	648	−817
Dav.	800	138	487	358	+ 94
Gow.	900	138	930	854	−746
McK.	900	145	895	772	−622
Ash.	900	166	830	621	−385
Bow.	900	166	1132	476	−542
Mean	870	145	851	618	−442

* These figures include the water in the blood sample withdrawn (about 15 ml. in each experiment).

Ketosis and Water Balance.—In spite of a liberal water intake in only three of the twenty experiments were the subjects in positive balance for the whole period of walking and recovery (Table III). Both evaporative and urinary water losses were greater in the warm than in the cool experiments. Metabolic water was not significantly different.

TABLE IV.—AMOUNTS OF PROTEIN, CARBOHYDRATE AND FAT UTILIZED IN 2½ HR. WALKING AND 6 HR. RECOVERY *

Subject	Walking			Recovery		
	Protein g.	Carbohydrate g.	Fat g.	Protein g.	Carbohydrate g.	Fat g.
			A.—Warm environment (21–25° C.)			
Bar.	13	3	98	18	6	46
Cas.	5	66	83	22	48	29
Don.	11	47	69	22	15	31
Dow.	17	56	71	18	0	66
Gra.	6	146	46	21	83	16
Dav.	6	108	31	16	36	18
Gow.	9	71	60	15	24	29
McK.	9	92	50	19	137	0
Ash.	11	110	66	26	21	31
Bow.	8	79	69	31	36	25
Mean	10	78	64	21	41	29
			B.—Cool environment (8–14° C.)			
Bar.	3	0	100	22	6	53
Cas.	5	1	85	16	0	43
Don.	5	33	68	22	18	29
Dow.	11	72	75	27	0	43
Gra.	3	62	59	19	21	38
Dav.	5	17	66	18	0	44
Gow.	5	43	61	22	19	25
McK.	8	45	56	21	42	20
Ash.	10	45	70	28	30	28
Bow.	8	22	66	21	96	12
Mean	6	34	71	22	23	34

* Statistical analysis: according to "Chi Square" analysis, warm was greater than cool in walking for carbohydrate at the 1 per cent probability level, and for protein at 6 per cent; fat was less at the 6 per cent level. In recovery, carbohydrate was greater in the warm at the 6 per cent level, and no differences existed for protein and fat.

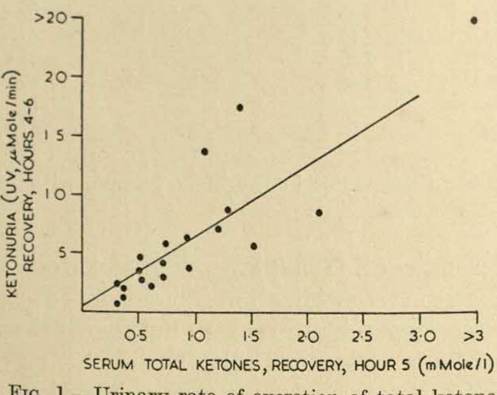

FIG. 1.—Urinary rate of excretion of total ketone bodies (μ-mole/min.) during fifth and sixth hours of recovery compared with serum concentration (m-mole/l.) at the fifth hour. The line was fitted by the method of least squares; the correlation coefficient was $+0{\cdot}68$, and the regression equation: $UV = (6{\cdot}1 \times \text{serum concentration}) + 0{\cdot}3$.

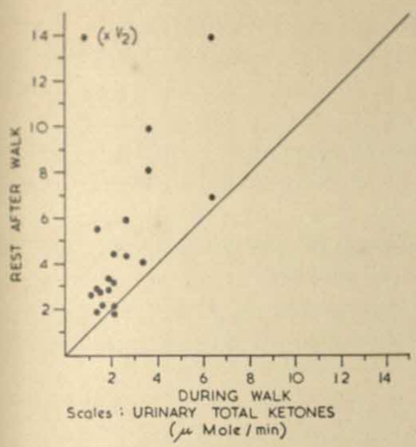

FIG. 2.—Ketonuria during a walk of 2½ hr. and a recovery of 6 hr. compared. Both scales: rate of excretion of total ketone bodies, μ-mole/min. Oblique line: equal values for walk and recovery. By the "Chi Square" test, the values during the walk were less than those during recovery at the ·001 per cent probability level.

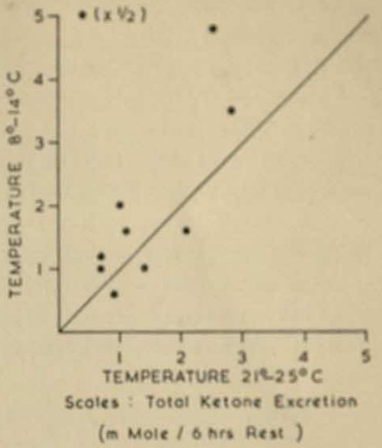

FIG. 3.—Effect of environmental temperature on ketonuria during 6 hr. of recovery after a 10-mile walk. Both scales: m-mole/6 hr. Ordinate: cool (8–14° C.). Abscissa: warm (21–25°). Oblique line: equal values for the warm and cool environments. By the "Chi Square" test values for warm were less than values for cool at a probability of 5 per cent.

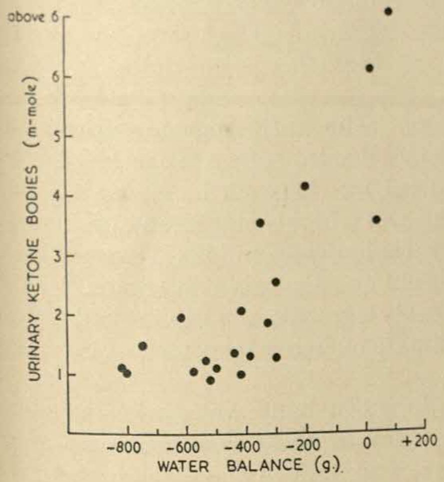

FIG. 4.—Ketonuria compared with the water balance during the combined periods of 2½-hr. walk and 6-hr. recovery. Ordinate: excretion of ketone bodies (m-mole). Abscissa: complete water balance (g.). A least square regression equation was log (ketonuria in m-mole) = + 0·88 (water balance in kg.) + 0·64, with an r of + 0·80.

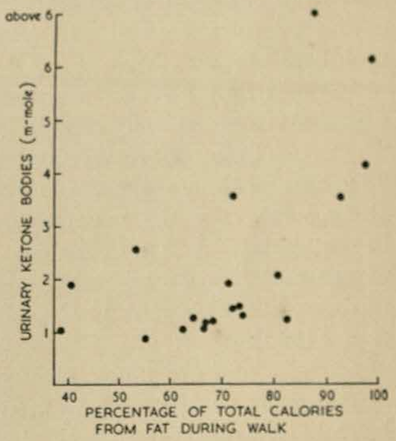

FIG. 5.—Ketonuria during 2½-hr. walk and 6-hr. recovery compared with percentage of calories in the metabolic mixture derived from fat. Ordinate: excretion of ketone bodies (m-mole). Abscissa: percentage of total calories derived from fat. A least square regression equation was log (ketonuria in m-mole) = + 1·11 (proportion of calories from fat) − 0·50, with an r of + 0·62.

Fig. 4 shows that the combined water balance for the walk and recovery was correlated with urinary ketones excreted during the same time. When the negative water balance was greater than − 400 g., significant ketosis did not develop. The larger rates of excretion of ketones were associated with positive water balance.

Ketosis and the Metabolic Mixture.—Table IV shows that during both the walk and recovery, fat usually provided the major portion of the calories. Fig. 5 shows the relation between ketonuria and the percentage of total calories derived from fat during the walk. When fat provided 82 per cent or more of the calories, ketonuria was always well marked.

There was no correlation between the percentage of the total calories derived from fat during recovery and ketonuria.

DISCUSSION

The Courtice-Douglas Effect as a General Phenomenon.—Our results are consistent with the hypotheses that during exercise the liver produces increased quantities of acetoacetic acid. These are readily oxidized by the working muscles during the walk and so ketones do not accumulate in the blood or appear in the urine in increased amounts. When exercise ceases, the muscles no longer utilize large amounts of acetoacetic acid and the ketone bodies can then accumulate, if liver production continues at a rapid rate. The Courtice-Douglas effect is therefore probably a measure of the rate of production of acetoacetic acid by the liver. The current view, as expressed by Campbell and Best [1956], is that this is controlled by fat-mobilizing factors.

Ketosis and Environmental Temperature. The antiketogenic effect of a warm environment may be attributable to mobilization of extra carbohydrate. The mean amount of carbohydrate mobilized for the walk in the warm was 78 g. and for the walk in the cool 34 g. This was a highly significant difference. During recovery the mean amounts of carbohydrate mobilized were 41 g. in the warm and 23 g. in the cool, which is only a suggestive difference. This excess mobilization of carbohydrate probably explains the diminished ketonuria and may account for the effects of climate on ketosis reported by Sargent *et al.* [1958].

Ketosis and the Water Balance.—The losses of body water are probably attributable to the starvation during the day of the experiment and may have been aggravated by the low content of sodium in the diet on the two previous days. We have no explanation of the enormous individual variations (from − 817 to + 94 g.). There is no significant difference between the mean balances in the warm and cool environments. Table IV shows that the large negative water balances were caused by large urinary outputs. These often occurred during recovery although the walk had previously caused slight dehydration.

The observation that dehydration is an antiketogenic factor appears to be quite new. It is, however, in line with the finding that limiting fluid intake for 2 weeks reduces nutritional ketosis [Sargent *et al.* 1958]. We have no profitable speculations on the mechanisms responsible for this correlation or on its biological significance.

Ketosis and the Metabolic Mixture.—The composition of the metabolic mixture has been determined from the respiratory data and the urinary nitrogen. This assumes an unchanging acid-base balance and regular breathing. In these experiments the subjects were never in an absolutely steady state; in all, the R.Q.s fell during the walk and some accumulation of ketone bodies occurred during recovery. These changes took place slowly over hours and for practical purposes the subjects can be considered to have been steady during the 5–10 min. when the respiratory measurements were made.

The significance of errors due to irregular breathing is more difficult to assess. All the subjects had had previous experience of respiratory apparatus in their practical classes and there was no obvious evidence of irregular breathing during the sampling periods. Most of the figures in Table IV are based on the mean of five measurements walking or of six during recovery. In all experiments during the walk, the figures for the R.Q.s fell slightly and usually evenly; there is every presumption that the mean figure for O_2 consumption and CO_2 production are satisfactory for calculating the metabolic mixtures. Respiratory data obtained during walking are always more uniform than data obtained at rest and we have every confidence in their use to calculate the metabolic mixture during the walk. The data during recovery appear useful, though certainly less reliable than those during the walk.

Shaffer [1921] and Woodyatt [1921] calculated ketogenic/antiketogenic ratios from the components of diets and metabolic mixtures and used them to predict the ketogenicity of a regimen. It can be shown that both of their ratios as well as the percentage of total calories derived from fat reduce to very similar mathematical functions of CO_2 produced, O_2 consumed and urinary nitrogen. With our data the same conclusions were reached with all three calculations and we have chosen to present the results in terms of percentage of total calories derived from fat, as being the simplest and most in accord with current usage.

Ketosis was correlated with the percentage of the total calories derived from fat during the walk, but not during recovery. The metabolic mixture during the walk is thus of value in predicting subsequent ketosis. This might indicate that a fat-mobilizing factor produced during the walk contributed to ketosis during recovery.

Ketosis as a Correlate of a Combined Function of the Water Balance and the Metabolic Mixture.—Figs. 4 and 5 show that the rate of urinary excretion of ketone bodies is correlated with both the water balance and the metabolic mixture during the walk. A better correlation is presented in fig. 6 where rates of urinary excretion are plotted against a combined function of these two factors. The effects of the water balance and the percentage of total calories derived from fat were shown to be additive. The mathematical transformation of figs. 4 and 5 is:

urinary ketone bodies during walk and recovery =

$$100\left(\frac{\text{water balance during walk and recovery}+1200}{1200-407}+\frac{\text{per cent of total calories from fat during walk}}{72\cdot 1}\right).$$

The figures 407 and 72·1 are the modes of the respective series. The combined function (fig. 6) thus calculated gives less scatter than either of the components alone (figs. 4 and 5), as the correlation coefficients prove.

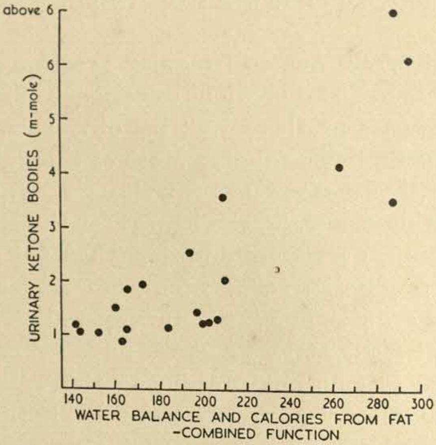

FIG. 6.—Ketonuria during $2\frac{1}{2}$-hr. walk and 6-hr. recovery compared with a combined function of the water balance during walk and recovery and percentage of total calories in the metabolic mixture derived from fat during the walk. Ordinate: excretion of ketone bodies (m-mole). Abscissa: combined function (see text). A least square regression equation was log (ketonuria in m-mole)

$$= +0\cdot60\left(\frac{\text{combined function}}{100}\right) - 0\cdot90,$$

with an r of +0·95.

The physiological meaning is probably that rates of urinary ketone excretion depend upon both the state of hydration and the metabolic mixture and not on either alone. The residual scatter in fig. 6 suggests that other undetected factors also exist.

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THE EFFECTS OF INFUSIONS OF ADRENALINE AND NORADRENALINE ON THE HEART RATE AND ARTERIAL BLOOD PRESSURE OF CONSCIOUS DOGS.

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In conscious dogs infusion of adrenaline at 0·03–0·2 μg./kg./min. caused an increase in the heart rate and very little change in arterial blood pressure.

Noradrenaline infused in conscious dogs at 0·1–0·5 μg./kg./min. caused a decrease in heart rate and an increase in systolic and diastolic arterial blood pressure.

The dose of adrenaline which produced an increase in heart rate was thus about one-third that of noradrenaline which produced a decrease in heart rate and an increase in arterial blood pressure.

Descriptions of the actions of adrenaline and noradrenaline are generally based on experiments upon anæsthetized animals, usually cats. The actions of these substances on the circulation have been studied in conscious human subjects and the results of such experiments have been reviewed by Barcroft and Swan [1953]. Although most of the effects on human subjects are similar to actions previously described in animals, some effects are especially prominent. Clearly, the true physiological functions of adrenaline or noradrenaline circulating in life are more likely to be reflected by intravenous infusions into conscious animals than by rapid injections into anæsthetized animals of doses, often apparently very large. There is, however, no systematic account in the literature of the action of adrenaline and noradrenaline on the circulation of conscious animals other than man, and this gap is partly filled by the results to be presented in this paper.

METHODS

The results have been obtained from three bitches during experiments in which the effects of adrenaline and noradrenaline on kidney function were also observed. The changes in the urine are reported in another paper [O'Connor, 1958]. At the beginning of each individual experiment a catheter was passed into the bladder and, also, a dose of 0·9 per cent sodium chloride, water or 2–5 per cent urea, was given by stomach tube. The animal was placed on its side on a warm table and, being well accustomed to the procedure, lay quietly for the duration of the experiment. The infusion cannula was inserted into the malleolar vein and infusion of saline begun. Recordings of the blood pressure and heart rate were obtained about every 3 min. for a preliminary period of 20 min. or more; at the same or more frequent intervals during an infusion of adrenaline or noradrenaline lasting 15–50 min.; then the infusion cannula was withdrawn and observations continued for at least another 30 min.

Intravenous infusions were given through a polythene cannula of external diameter 1 mm., bore 0·5 mm., inserted into the malleolar vein and threaded forward about 25 cm. so that the tip of the cannula was in the femoral or iliac vein. The cannula was inserted by using half of a venesection needle cut longitudinally as an introducer [Mitchell, 1952]. The bevelled end of the cannula was fitted within the bevelled, sharp point of the half-needle and the composite "needle" inserted into the vein through a small area anæsthetized with procaine hydrochloride (2 per cent); the half-needle was then slipped back and the cannula threaded forward. The same vein was used repeatedly without complications. The cannula was connected by a two-way tap to two 20-ml. syringes mechanically driven to deliver, usually, 0·26 ml./min. Each syringe contained 0·85 per cent sodium chloride, and one, in addition, the drug; the dose to be infused in each experiment was obtained by an appropriate concentration in the infusion fluid. The final dilution was made up immediately before the experiment, using adrenaline (BDH) dissolved in a small quantity of N/10 hydrochloric acid and noradrenaline bitartrate (Bayer); doses in the text are given in terms of the bases. Aseptic precautions were taken in preparing the solutions and in giving the infusions.

Arterial blood pressure was measured in two of the animals ("Skewbald" and "Floss") by the indirect method described by O'Connor [1955]. Systolic and diastolic pressures were determined by means of an oscillometer connected to a pressure cuff applied to the femoral artery previously enclosed in a skin loop, and the heart rate was counted from the oscillometer records. The mean arterial blood pressure was calculated as the diastolic pressure plus one third of the pulse pressure. In the third animal ("Black Sue") a loop was prepared as in the other two, but after about 2 months an abscess developed at the lower end of this loop and the artery returned to a subcutaneous position as the abscess healed. A metal clip was constructed to hold a rubber compression bag and one limb of this clip slipped under the artery by invaginating the loose skin of the groin. When the bag was inflated, pressure was applied to the artery from one side against the metal clip. Satisfactory oscillometer records were obtained, and the changes in blood pressure recorded by this device from "Black Sue" were similar to those in the other animals by a cuff completely surrounding the artery in its skin loop.

Electrocardiograms were recorded by leads from the right forelimb, the left forelimb and from the right or left hindlimb. Contact was made by impregnating the short fur of the paw with electrode jelly and bandaging in place a sheet of soft metal from which the leads were taken to a lead selector switch. From here the current was fed, after suitable amplification, to a single channel of a four channel ink-recorder (Southern Instruments Limited).

Results

Adrenaline.—Fig. 1 shows three experiments with "Skewbald" in which adrenaline was infused at 0·034, 0·073 and 0·17 μg./kg./min. In each the heart rate increased by 50–60 beats/min., and the tachycardia persisted throughout the period of the infusion even in instances, not illustrated, when the infusion was continued for 50 min. The oscillometer records and electrocardiograms showed no sign of cardiac irregularity when the rates were high. Thus, in fig. 2, the well marked sinus arrhythmia of the control record was lost during the infusion of adrenaline but the complex of each beat was unaltered. In each of nineteen experiments on "Skewbald" over a period of 4 years, adrenaline always produced a similar tachycardia. Likewise, in the other two dogs, adrenaline always produced an increase in heart rate. In eleven experiments on "Black Sue" there was an increase of 20–35 beats/min.; in nine experiments on "Floss" the increase in heart rate varied from 15–60 beats/min.

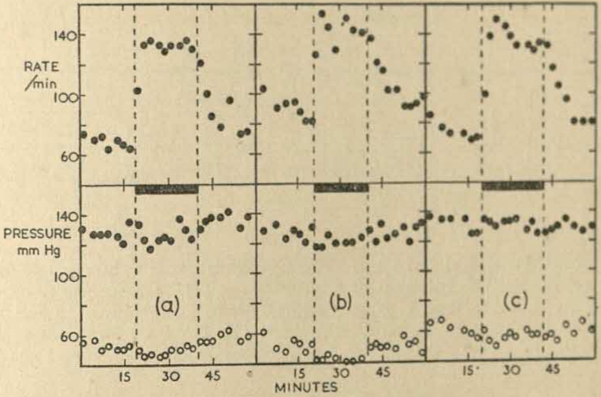

FIG. 1.—"Skewbald", 15 kg. The effect of the infusion of adrenaline at (*a*) 0·034, (*b*) 0·073 and (*c*) 0·17 μg./kg./min. Abscissæ; time in min. Ordinates: above, pulse rate in beats per min.; below, systolic and diastolic blood pressures in mm. Hg. Adrenaline was infused during the period indicated by the rectangles.

The dose of adrenaline needed to produce tachycardia was of the order of 0·03 μg./kg./min. In the upper half of fig. 3*a* the increase in heart rate is plotted against the dose of adrenaline infused in thirty-nine experiments on the three dogs; always a large increase in heart rate occurred if the dose was greater than 0·03 μg./kg./min. The full increase characteristic of each group of experiments was produced by infusion of 0·05 μg./kg./min. and infusions of doses up to 0·2 μg./kg./min. produced the same effect.

The arterial blood pressure was little altered by adrenaline (fig. 1); the systolic and diastolic pressure was determined in fifteen experiments on the three animals and, always, the values obtained during the infusion were within 12 mm. Hg of the values before and after the administration of adrenaline. Fig. 1*b* is an example where there appeared to be a fall chiefly in diastolic pressure during the infusion, but the apparent changes were never more definite than in this example. In the lower frame of fig. 3*a* any changes in mean blood pressure are plotted against the dose of adrenaline infused,

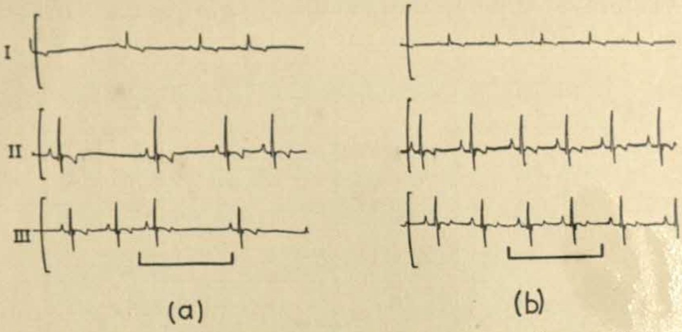

Fig. 2.—"Skewbald", 15 kg. Electrocardiograms taken (*a*) before and (*b*) during the infusion of adrenaline at 0·17 μg./kg./min. Dog lying on R. side; lead I, from R. forelimb to L. forelimb; Lead II, from R. forelimb to R. hindlimb; lead III, from L. forelimb to R. hindlimb. Vertical bars are 2 millivolts; horizontal bars 1 sec.

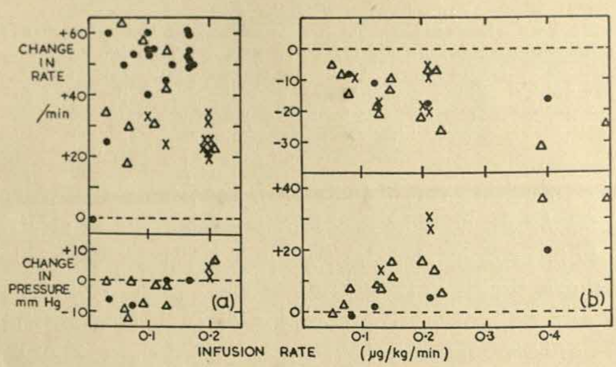

Fig. 3.—The effects of different rates of infusion of (*a*) adrenaline and (*b*) noradrenaline on three dogs. Experiments on "Skewbald" are shown by circles, on "Floss" by triangles and on "Black Sue" by crosses. Abscissæ; rate of infusion (μg./kg./min.). Ordinates: above, change in pulse rate (beats per min.); below, change in mean arterial blood pressure (mm. Hg).

and from this it would appear that doses of about 0·1 μg./kg./min. produced a small fall. Such changes were always less than 12 mm. Hg and are of doubtful significance in view of the limitations of the indirect method of recording the pressure.

The effects of adrenaline in conscious dogs may thus be summarized as increase in heart rate with very little change in arterial blood pressure.

Noradrenaline.—Fig. 4*a*, *b* and *c* show the results of experiments in which noradrenaline was infused in "Floss" at 0·08, 0·15 and 0·40 μg./kg./min.

The results in fig. 4*b* and *c* are typical of experiments in which noradrenaline was infused in effective doses in the three animals; there was an increase in both systolic and diastolic blood pressure of up to 35 mm. Hg, together with a fall in heart rate of as much as 30 beats/min. In the conscious dog, as in the human subject, noradrenaline thus produced increase in blood pressure and slowing of the heart rate.

Fig. 3*b* shows the effect of noradrenaline in twenty-three experiments on the three animals plotted against the dose infused. The smallest dose of noradrenaline to produce a slowing of the heart and increase in blood pressure was 0·1–0·15 μg./kg./min., which was three times the dose of adrenaline needed to produce tachycardia.

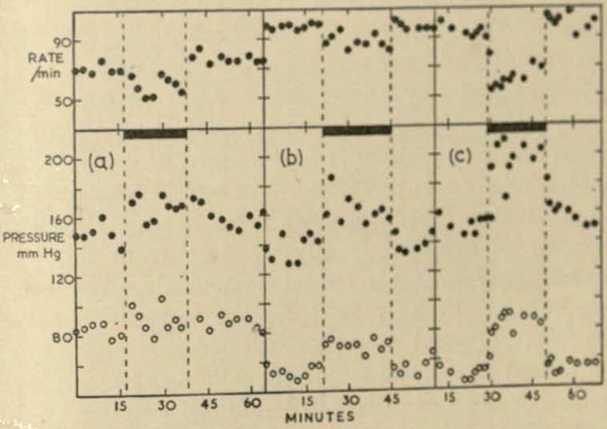

FIG. 4.—"Floss", 20 kg. The effect of the infusion of noradrenaline at (*a*) 0·08, (*b*) 0·15 and (*c*) 0·4 μg./kg./min. Abscissæ; time in min. Ordinates: above, pulse rate in beats per min.; below, systolic and diastolic blood pressure in mm. Hg. Noradrenaline was infused during the period indicated by the rectangles.

General Effects.—The infusion of adrenaline at doses up to 0·2 μg./kg./min. and of noradrenaline up to 0·5 μg./kg./min. produced no restlessness in the dogs. Accustomed to lie quietly on the table, they continued to do so without any indication of the feeling of anxiety which is experienced by human subjects during the infusion of adrenaline [Barcroft and Swan, 1953]. There was no obvious change in respiration, but counts of the respiratory rate were not made.

DISCUSSION

The effects of adrenaline and noradrenaline described above are qualitatively similar to the effects produced by the infusion of these substances in human subjects, summarized by Barcroft and Swan [1953, fig. 9, 1]. During infusions of adrenaline in the conscious dogs the increase in heart rate was, however, much greater and more persistant than in human subjects. In the latter tachycardia was maximal about 2 min. after the start of an infusion but then fell and, for the duration of the infusion, remained at a value of about 10 beats/min. above the resting rate [Allen, Barcroft and Edholm, 1946]. In conscious dogs tachycardia persisted unchanged throughout the period of infusion. The changes in arterial blood pressure produced by adrenaline in dogs were much smaller than those found in the human subject by Allen *et al.* [1946], and the increase in systolic and decrease in diastolic pressure illustrated by Barcroft and Swan [1953] was not observed in our experiments on dogs. The effects of infusions of noradrenaline in conscious dogs were almost identical with those described in man by Barcroft and Konzett [1949].

Our results differ in some respects from the few previous reports of the effects of infusion of these amines in conscious dogs. Pickford and Watt [1951] infused adrenaline and noradrenaline at rates of 2–10 μg./min. in dogs weighing 8·5–17 kg. and found that both caused slowing of the heart rate; after atropine adrenaline caused an increase in heart rate while noradrenaline caused a decrease. The experiments of Pickford and Watt were conducted with the animals standing and not lying as in our experiments, and the doses of adrenaline were larger than those we have used. Harries [1956] found complex changes in heart rate, usually slowing, from the infusion of noradrenaline (0·3–1·2 μg./kg./min.) in conscious dogs already receiving histamine (0·15–1·5 μg./kg./min.). Blake [1955] mentions that there was tachycardia and a transient fall in arterial blood pressure in three experiments in which adrenaline was infused in conscious dogs at 0·28 μg./kg./min.

That the effective dose of adrenaline may be smaller than that of noradrenaline is not indicated in the literature. In human subjects, Allen *et al.* [1946] investigated the effects of the infusion of adrenaline mostly at doses of 10 μg./min. for 10 min. A few examples are given which indicate that 1 μg./min. had no effect; in one example 3 μg./min. produced an increase in heart rate equal to that produced by 10 μg./min., and the tachycardia produced by 20 μg./min. appeared no greater. Barcroft and Konzett [1949] infused adrenaline for shorter periods (3 min.); again, in one example, the tachycardia produced by 5 μg./min. was as great as that produced by 10, 20 or 30 μg./min. On the other hand, the increase in blood pressure and slowing of the heart rate produced by the infusion of noradrenaline at 5 μg./min. was less than that produced by 10 μg./min., and the effect of 20 μg./min. was still greater. It thus appears possible that systematic investigation of the dose-response relationships in human subjects might reveal that the dose of adrenaline needed to produce tachycardia when infused is less than that of noradrenaline required to produce bradycardia and increased blood pressure. Goldenberg, Pines, Baldwin, Greene and Roh [1948] stated that infusion of a mixture of equal parts of noradrenaline and adrenaline showed predominantly the effects of adrenaline, while de Largy, Greenfield, McCorry and Whelan [1950] found that infusion of a mixture containing three to eight parts of noradrenaline to one part of adrenaline had no effect on the heart rate of human subjects. The relative activity of the two amines in conscious animals could be of importance in assessing the physiological rôle of small amounts of the amines liberated into the circulation in normal life.

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THE EFFECT ON URINARY VOLUME AND COMPOSITION OF THE INGESTION OF 0·9 PER CENT SODIUM CHLORIDE AND OF OCCLUSION OF THE CAROTID ARTERIES.

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In conscious dogs, changes in the urine following the ingestion of 0·9 per cent sodium chloride presented two different pictures:

(*a*) If no previous doses of sodium chloride had been given, there was an increase in sodium excretion from the resting value of 0·002 m.equiv/min. up to 0·02 m.equiv/min. because of increased sodium concentration in the urine, without great increase in the urinary volume. Potassium excretion was also increased.

(*b*) If sodium excretion had been raised above 0·02 m.equiv/min. by previous doses of saline, there was a much greater increase in sodium excretion (up to 0·3 m.equiv/min. or more) mainly due to increased urinary volume without any large change in the sodium concentration. Potassium excretion also increased, but no more than in (*a*) above.

The rate of excretion of sodium has been plotted against the fall in the total solid content of the plasma, used as a convenient indicator of the changes in plasma proteins. Fall of the plasma solids by 0·6 g./100 g. below the resting value was associated with a response of type (*a*) above in which the rate of excretion of sodium increased by 0·002–0·005 m.equiv/min. for each fall of 0·1 g./100 g. in plasma solids. Further fall of plasma solids was associated with response (*b*) above, and the sodium excretion increased by 0·05–0·09 m.equiv/min. for each fall of 0·1 g./100 g. in plasma solids.

Occlusion of both carotid arteries increased the rate of excretion of sodium. If the existing rate of excretion of sodium was low as in (*a*) above, increased excretion of sodium during carotid occlusion was largely due to the increased concentration of sodium in the urine; if the rate was higher as in (*b*) above, the increase on carotid occlusion was due to increased volume of the urine. Carotid occlusion also increased the excretion of potassium. The effect of carotid occlusion was similar to the effect of a fall of 0·1–0·2 g./100 g. in plasma solids.

The effects of the ingestion of saline or carotid occlusion are attributed to the direct effect on the kidney of dilution of the plasma proteins or increased arterial blood pressure. Comparison is made with the renal effects of the infusion of adrenaline and partial obstruction of the renal artery and it is suggested that all four circumstances produce their effect by alteration of factors affecting glomerular filtration. A diagram is presented showing the changes in urine volume and excretion of sodium, potassium and urea which have been attributed in this paper to changes in glomerular filtration rate.

INTRODUCTION

IN previous experiments [O'Connor, 1955 *b*] isotonic solutions of sodium chloride were administered to conscious dogs, which had already been given priming doses of sodium chloride on the day of the experiment and on preceding days. The effect of ingested saline on renal function was thus described under conditions when the rate of excretion of sodium was already raised and a relationship drawn between changes in the rate of excretion of sodium and dilution of the plasma solids (representing plasma protein concentration). In discussion, it was pointed out that this relationship was probably of great importance in the maintenance of sodium balance at high salt intakes, but that separate investigation was needed of the factors controlling sodium excretion at normal and low levels of sodium intake. In this paper a description is given of the renal response to small doses of 0·9 per cent sodium chloride administered to animals maintained on the normal laboratory diet without any preliminary doses of saline. Again it has been possible to relate rate of excretion of sodium to dilution of the plasma solids, so that, in conjunction with the previous work, a relationship between these two factors has been plotted over a range of sodium excretion from 0·002–0·5 m.equiv/min.

In the previous work O'Connor [1955 *b*] found that occlusion of both carotid arteries produced increased excretion of sodium, and this was explained as the direct effect on the kidney of the raised arterial blood pressure. The effects of carotid occlusion when the rate of excretion of sodium was low are also described in this paper and compared with the effects of ingestion of small doses of 0·9 per cent sodium chloride.

The results in this paper, the renal effects of infusions of adrenaline [O'Connor, 1958] and the effects of obstruction of the renal artery [*e.g.* Mueller, Surtshin, Carlin and White, 1951] have many features in common and appear to be due to small changes in glomerular filtration. In the discussion, a provisional account is suggested of the changes in urine flow and composition which may be attributed to small changes in glomerular filtration under different experimental conditions. It appears that small changes in glomerular filtration produce larger changes in sodium excretion than in the excretion of any other urinary constituent.

The results have been presented briefly to the Physiological Society [O'Connor, 1958 *a*].

METHODS

All of the experiments were performed on trained, conscious bitches. At a preliminary aseptic operation the perineum was split to expose the urethra and so make catheterization easy. Also at aseptic operations the carotid arteries were enclosed in van Leersum skin loops and in some of the animals femoral artery loops were also provided for the measurement of arterial blood pressure by the method of O'Connor [1955 *a*].

The animals were fed about 200 g. of dog biscuit and about 100 g. of meat per day, containing about 15 m.equiv. of sodium and 22 m.equiv. of potassium. The ration was fed about 4 P.M. each day and was usually consumed within an hour. The animals were exercised on the roof each morning. There was free access to water, but the animals usually drank at the time of the meals or after exercise. During and for an hour before an experiment water was not offered.

For each experiment, a self-retaining catheter was inserted into the bladder and the animal was then placed on its side on a warmed table, and being well accustomed to the procedures, lay quietly for the duration of the experiment. A rest period of 25 min. was allowed before any urine collection was made, and this was extended if the animal had not settled quickly or if the pulse rate had not fallen below 90 beats per min. Doses of saline or water were given by stomach tube without disturbing the animal from the lying position.

In most of the experiments the rate of flow of urine was low. The bladder was, therefore, washed out during the last minute of each collection period by two volumes of 10 ml. of distilled water accurately measured from a burette. The volume of the urine was then determined as the difference between the collected volume and the volume of the wash. The amount of a urinary solid was determined by analysis of the collected fluid, and the urinary concentration calculated by dividing the amount excreted by the volume of the urine.

Blood samples of about 3 ml. were drawn by puncture of the carotid artery contained in a skin loop and collected into syringes moistened with 0·02 ml. of saline containing 20 units of heparin. The blood was immediately centrifuged at 900 *g* for 15 min. and the plasma separated for analysis.

Carotid occlusion was produced by surrounding the carotid loops by small pressure cuffs which could be connected to a large reservoir of air at a pressure of 250 mm. Hg. In some experiments the arterial blood pressure was measured by the method of O'Connor [1955 *a*].

Intravenous infusion of 0·85 per cent sodium chloride was used in a few experiments. A polythene cannula was inserted into the malleolar vein as described by Holgate and O'Connor [1958], and threaded forward so that the tip lay in the femoral or iliac vein. The infusion was delivered by a 200-ml. syringe, mechanically driven; the syringe and connecting tubing were kept at about 35° C. by the heat from an electric lamp but no accurate control of the temperature of the infusion was attempted.

Methods of Analysis.—Sodium and potassium in urine and plasma were determined by external standard flame photometer [EEL, Collins and Polkinhorne, 1952]. *Urinary chloride* was determined by the method of Wilson and Ball as described by Peters and Van Slyke [1932] and *urea* by the method of Scott [1940]. *Solid content of plasma* was determined by weighing, drying and reweighing about 0·5 g. of plasma. It was dried at 95–105° C. for 3 hr., after which there was no further weight loss.

RESULTS

The Effect of 0·9 per cent Sodium Chloride

Sodium Excretion.—When 200–400 ml. of 0·85–0·9 per cent sodium chloride was given by stomach tube the rates of excretion of sodium and chloride were always increased about equally, but the extent and manner of the increase was altered by prior administration of saline. In the example of Table I, no sodium salt had been administered in the preceding 7 days except the 15 m.equiv/day contained in the diet, and the volume and composition of the urine is shown during three periods—in line 1, before any dose; in line 2, 75–150 min. after 250 ml.; and in line 3, 73–113 min. after a second dose of 400 ml. of saline by stomach tube. The time course of the response to the first dose of saline is plotted in fig. 1*a*.

TABLE I.—THE EFFECT ON THE URINE OF 0·9 PER CENT SODIUM CHLORIDE BY STOMACH TUBE

Time	Volume ml./min.	Sodium		Potassium		Urea		Plasma solids g./100 g.
		m.equiv/ min.	M	m.equiv/ min.	M	m.equiv/ min.	M	
9.52–10.42 A.M.	0·08	0·0013	0·017	0·0021	0·026	0·100	1·25	8·61
NOON– 1.15 P.M.	0·12	0·017	0·14	0·0095	0·079	0·095	0·79	7·98
2.31– 3.11 P.M.	1·12	0·28	0·25	0·017	0·015	0·115	0·10	7·61

"Black Sue", weight 22 kg. At 10.45 A.M. 250 ml. and at 1.18 P.M. 400 ml. of 0·88 per cent sodium chloride was given by stomach tube. The figures under sodium, potassium and urea give the urinary excretion (m.equiv/min.) and urinary concentration (Molar). The animal was lying quietly on the table except from 1.15 to 2.00 P.M. when it was returned to its cage.

The response to the second dose of saline was as described when saline was given to animals primed by previous doses of saline [O'Connor, 1955 *b*]. Thus, in line 3 of Table I the rate of excretion increased sixteen fold compared with the rate in line 2, and this was mainly due to a nine-fold increase in the urinary volume, with only a small increase (× 1·8) in urinary sodium concentration; often there was no change or even a fall in sodium concentration in this type of response [O'Connor, 1955 *b*].

The first dose of saline, given without any priming dose, also produced increased sodium excretion (compare lines 2 and 1 of Table I); here, a thirteen-fold increase at a low level of sodium excretion involved only a small increase in the volume of the urine (× 1·5) but was largely due to an eight-fold increase in the concentration of sodium in the urine. This experiment is typical of twenty-three experiments on three dogs in which doses of 150–250 ml. of 0·85–0·9 per cent sodium chloride have been given by stomach tube without any priming doses of saline. The small increase in sodium excretion produced by infusion of saline in fig. 1*b* has exactly the same characteristics. The increases in sodium excretion recorded in Table I and similar experiments were far greater than any change which occurred over the same period when no saline was given. In six control experiments sodium excretion at NOON–1.0 P.M. was 0·7–1·5 times and at 2.30–3.30 P.M. 0·7–2·8 times the rate at 9.30–10.30 A.M.

Fig. 1*a* shows the time course of the sodium excretion following the first dose of saline in the experiment of Table I; the change in sodium concentration occurred rapidly after the test dose, so that the fastest excretion and the highest concentration of sodium in the urine was reached in the period 50–75 min. after the test dose; and thereafter excretion and concentration steadily diminished. Fig. 1*b* shows the time course of a small increase in sodium excretion following the intravenous infusion of 0·85 per cent sodium chloride at 2·33 ml./min. for 43 min.; again the sodium concentration increased without change in the volume and the highest values were recorded immediately after the infusion.

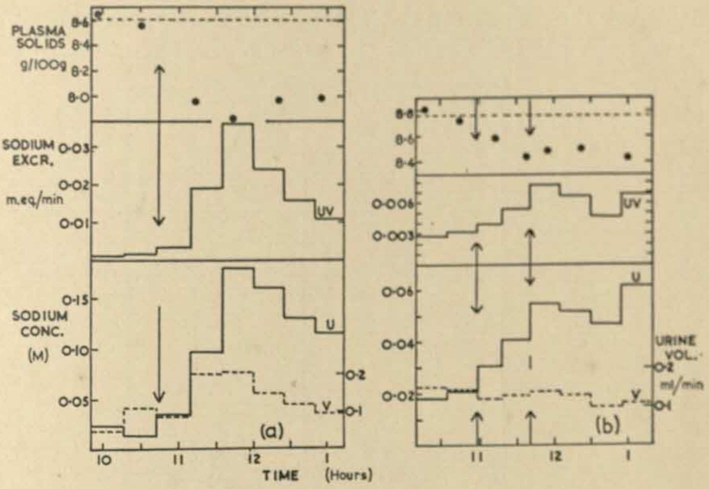

FIG. 1.—"Black Sue", weight 22 kg. Changes in the urinary excretion of sodium (*a*) following the administration of 250 ml. of 0·88 per cent sodium chloride by stomach tube at the time marked by the arrow, (*b*) following the infusion of 100 ml. of 0·85 per cent sodium chloride at 2·33 ml./min. during the period between the arrows. No priming doses of saline had been given. In each graph abscissæ, time (hours of the day). Ordinates: lower frame; dotted line V, urine flow (ml./min., scale on right); continuous line U, sodium concentration (molar, scale to left); centre frame, rate of excretion of sodium UV (m./equiv/min.); upper frame, total solids of plasma (g./100 g. water).

Relationship between Sodium Excretion and Plasma Solids.—Plasma from blood drawn from the carotid artery was analyzed in the experiments of fig. 1*a*, *b* and Table I and many similar experiments. The mean from twenty-five such experiments on three dogs showed no change in the plasma sodium following the ingestion or infusion of 0·85–0·9 per cent sodium chloride. The changes in the solids of the plasma given in Table I and the upper frames of fig. 1 are typical of the twenty-five experiments. In control experiments where no saline was given, plasma solids fell below the initial value by 0·04–0·08 g./100 g. at NOON–1 P.M. and by 0·05–0·15 g./100 g. at 2.30–3.30 P.M. As in the experiments on animals primed by doses of saline [O'Connor, 1955 *b*], the time course of the excretion of sodium followed closely the dilution of the plasma solids; in fig. 1 the delay appears to be less than 10 min. That changes in the concentration of sodium in the urine could occur so rapidly at urine flows of only 0·1–0·2 ml./min. means that the renal dead space in respect of sodium excretion is small, which is in accordance with the findings of Chinard and Enns [1955].

In fig. 2*a*, from the experiment of Table I and fig. 1*a*, the rate of excretion of sodium has been plotted against the plasma solid content in each urine collection period. The fall of plasma solids from 8·61 to 7·8–8·0 g./100 g. produced by the first dose of saline was accompanied by an increase in sodium excretion from 0·0013 to 0·04–0·017 m.equiv/min.; the second dose of saline caused plasma solids to fall to 7·6 g./100 g. and this was accompanied by a large increase in sodium excretion to about 0·28 m.equiv/min. A curve of the general shape of that suggested by the points in fig. 2*a* would best fit the data in sixteen of eighteen experiments with three animals, fig. 2*b* being another example. In fig. 3 the results of all of the experiments with "Black Sue" have been plotted on the same graphs; to meet the fact that the plasma solid values may differ from one day to another, the rate of excretion of sodium has been plotted against the fall in plasma solids below the initial value in each experiment. In four experiments 1 dose only of saline was given and in four 2 doses were given, so that twelve points were available for plotting, each showing the rate of excretion of sodium and the plasma solid content 1–2 hr. after a dose of saline. Except for the one point in fig. 3*a* all twelve lie close to the line drawn by eye in the figures; in the case of the aberrant observation, the fall in plasma solids after a second dose of saline was larger

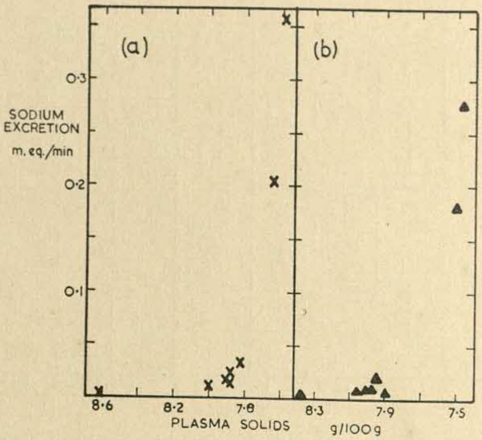

FIG. 2.—"Black Sue", weight 22 kg. The relationship between sodium excretion and the solid content of plasma in two experiments. Abscissæ; plasma solid content (g./100 g. water). Ordinates: rate of excretion of sodium (m.equiv/min.).

than was expected from the administered dose, and the rate of excretion of sodium did not rise in accordance with this unexpectedly large fall in plasma solids.

Experiments on two other animals have also given curves of the same shape as that of fig. 3. From twenty-five experiments on three animals, forty-three points were available for plotting in the manner of fig. 3, and thirty-four of them fell between two lines drawn parallel to the curve for each animal but 0·15 abscissa divisions (*i.e.* 0·15 g./100 g. plasma solids) to the right and left. The shape of the curves in the three animals may be compared by regarding them as consisting of two straight lines of different slope. In the lower part of the curve, representing the response to a single dose of saline, the slope was such that each fall of 0·1 g./100 g. in plasma solids was accompanied by an increase of sodium excretion of 0·002 m.equiv/min. ("Black Sue", fig. 3*b*), 0·0045 ("Skewbald") and 0·003 ("Tyke"). On the upper part of the curve, following a second dose of saline the same fall in plasma solids was accompanied by increases of sodium excretion of 0·07 m.equiv/min. ("Black Sue", fig. 3*a*), 0·085 ("Skewbald") and 0·05 ("Tyke"). The steep part of the curve began when the fall in plasma solids was greater than 0·5 g./100 g. ("Black Sue", fig. 3), 0·6 ("Skewbald") and 0·8 ("Tyke").

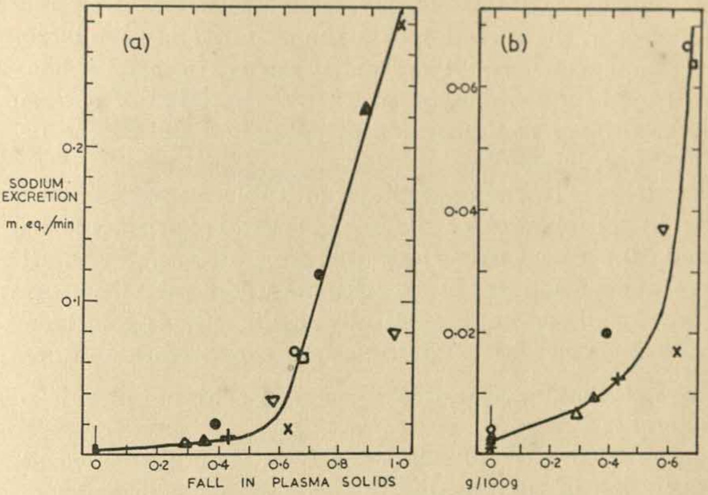

Fig. 3.—"Black Sue", weight 22 kg. The relationship between sodium excretion and plasma solid content in eight experiments on the one animal. A separate symbol is used for each experiment. Each point shows the fall in plasma solids below the initial value for that experiment (abscissæ, g./100 g.) and the rate of excretion of sodium (ordinate, m.equiv/min.) during a period of 40–75 min., 1 hr. or more after the administration of a dose of 0·85–0·9 per cent sodium chloride. (*a*) and (*b*) show the same data but the ordinate scale of (*b*) is more open to show the lower part of the curve more clearly.

Potassium excretion, is also shown in Table I; in this experiment potassium excretion increased after the first dose of saline to 4·5 and after the second dose to eight times the initial rate. These increases were rather larger than those usually observed; in the mean of fourteen experiments on two dogs potassium excretion after the first and second doses of saline was 2·7 ± 0·5 (standard error of mean) and 6·1 ± 1·5 times the initial value for the individual experiment. Some increase in potassium excretion also occurred in control experiments in which the same experimental routine was followed but no saline was given; at NOON–1 P.M. potassium excretion was 1·25 ± 0·22 (7) times and at 2.30–3.30 P.M. was 2·7 ± 0·8 (4) times the rate at 10 A.M. Probably, therefore, ingestion of 0·9 per cent sodium chloride did cause an increase in the excretion of potassium, but this was much smaller than the increase in the excretion of sodium. Certainly the large increase in sodium excretion which resulted from a second dose of saline was never accompanied by a comparable increase in the excretion of potassium, and at this stage the urinary concentration of potassium fell as the urine volume increased (Table I, line 3).

Urea Excretion.—In the experiment of Table I urea excretion was very little altered by the ingestion of saline. In control experiments urea excretion fell by about 10 per cent over the period from 10 A.M.–3 P.M. The fall was less or there was a small rise in experiments where saline was given, but the difference was small and not statistically significant. Again increasing urine volume was associated with a fall in urinary concentration of urea (Table I).

Responses to the ingestion of 0·9 per cent sodium chloride were thus of two characteristic forms:

(1) If no previous dose of saline had been given, saline caused increased excretion of sodium due to increased concentration of sodium in the urine without great change in the urinary volume or the rate of excretion of urea. There was probably a small increase in the excretion of potassium. The increase in sodium excretion was 0·002–0·005 m.equiv/min. for each fall of 0·1 g./100 g. in plasma solids.

(2) If from previous administration of saline, the plasma solids were reduced 0·6 g./100 g. or more below the initial value, administration of saline caused a much greater increase in sodium excretion mainly due to increased volume of the urine, the sodium concentration changing little. Sodium excretion was then increased by 0·05–0·09 m.equiv/min. for each fall of 0·1 g./100 g. in plasma solids. There was still only a small increase in potassium excretion.

The Effect of Occlusion of the Carotid Arteries

Sodium Excretion.—O'Connor [1955 *b*] found that high rates of excretion of sodium (0·05 m.equiv/min. or more) were increased by occlusion of both carotid arteries and this was attributed to the effect on the kidney of the increased arterial blood pressure. Fig. 4*a* and *b* show two examples of the effect of carotid occlusion at lower rates of excretion of sodium. In fig. 4*a* the resting rate of excretion of sodium of 0·002 m.equiv/min. was increased during occlusion to 0·004 m.equiv/min., and this was typical of eight similar experiments on two dogs. To obtain urine collections of sufficient duration occlusion was maintained in these experiments for as long as 75 min. The increase in blood pressure was of the order of 35–50 mm. Hg as previously described by O'Connor [1955 *a*, *b*], and was maintained throughout the period of occlusion. In fig. 4*b* the resting rate of excretion of sodium was higher (0·037 m.equiv/min.) and was increased to 0·098 m.equiv/min. during carotid occlusion, and this also is typical of seven experiments in which the resting sodium excretion at the time of carotid occlusion was 0·01–0·05 m.equiv/min. Similar changes in chloride excretion also occurred.

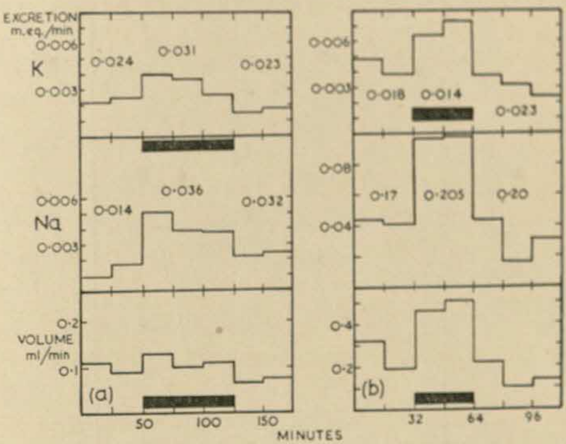

FIG. 4.—"Black Sue", weight 22 kg. The effect of occlusion of both carotid arteries (*a*) without any priming dose of saline and (*b*) 3 hr. after 300 ml. of 0·9 per cent sodium chloride by stomach tube. Abscissæ; time in minutes. Ordinates: lower frame, urine volume (ml./min.); middle frame, sodium excretion (m.equiv/min.); upper frame, potassium excretion (m.equiv/min.). The figures within the frames give the urinary concentration (Molar) of sodium and potassium.

It has been shown above that ingestion of saline caused increased sodium excretion mainly by increase in the volume of the urine when sodium excretion was high, and mainly by increase in the sodium concentration of the urine when the rate of excretion was low. Carotid occlusion produced similar effects. In experiments at rates of excretion of sodium above 0·05 m.equiv/min. [*e.g.* O'Connor, 1955 *b*, fig. 8] carotid occlusion increased sodium excretion by means of increased volume of the urine with usually no change or a small fall in the urinary sodium concentration; fig. 6*a* of this paper shows a further example. In experiments where the rate of excretion of sodium was lower (*e.g.* figs. 4*a*, *b* and 6*c*) carotid occlusion increased the urine flow a little in all instances but there was also an increase in sodium concentration, and the increase in sodium excretion was due to the combination of these two factors.

In fig. 5 quantitative comparison is made between ingestion of saline, and carotid occlusion as causes of increased excretion of sodium. The continuous line in fig. 5 is redrawn from fig. 3*a* and expresses the relationship between the rate of excretion of sodium and the solid content of the plasma in experiments in which saline was given to "Black Sue" with the mean arterial blood pressure about 100 mm. Hg in the absence of carotid occlusion. The results of the carotid occlusions have been plotted as in the following example. In the experiment of fig. 4*b* carotid occlusion increased the sodium excretion from 0·037 to 0·098 m.equiv/min.: in plotting this experiment in fig. 5, it was first seen from continuous line that excretion at 0·037 m.equiv/min. corresponds to plasma solid concentration of 7·68 on the arbitrary abscissa scale; and so the occlusion experiment was plotted as 7·68, 0·098. The points from eleven experiments on this dog shown on the graph, and one not shown in which sodium excretion was increased from 0·23 to 0·33 m.equiv/min. by carotid occlusion, all lie close to the dotted line drawn 0·12 abscissa divisions to the left of the continuous line, and this new line thus represents the relationship between plasma solid concentration and rate of excretion of sodium when mean arterial blood pressure was raised to about 136 mm. Hg

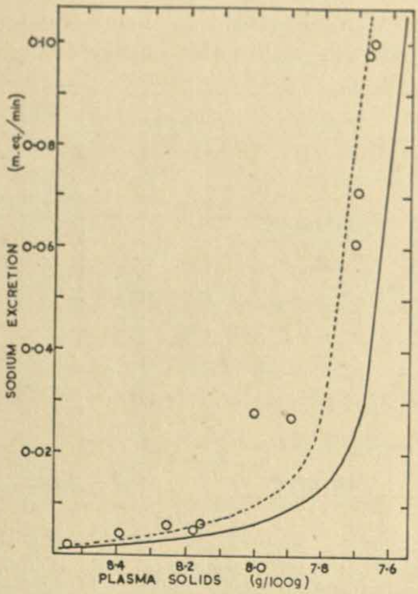

FIG. 5.—"Black Sue", weight 22 kg. Comparison of carotid occlusion and dilution of plasma solids as means of producing increased sodium excretion. The continuous line shows the relationship between plasma solid content (abscissæ; g./100 g.) and rate of excretion of sodium (ordinates: m.equiv/min.) at the normal mean arterial blood pressure of about 100 mm. Hg (replotting of fig. 3). The circles and dotted line, plotted as described in the text, show the relationship during occlusion of both carotid arteries with mean arterial blood pressure raised to about 136 mm. Hg.

as the result of carotid occlusion. At both high and low rates of excretion of sodium the effect of carotid occlusion was equal to that expected from a fall of 0·12 g./100 g. in plasma solids. In a second dog, "Skewbald", which has also been investigated at both low and high rates of excretion of sodium, carotid occlusion produced a rise of about 50 mm. Hg in arterial blood pressure and produced increased sodium excretion equal to the effect of a fall of 0·2 g./100 g. in plasma solids.

Potassium Excretion.—In twenty experiments on three dogs, carotid occlusion always produced an increased excretion of potassium. In experiments like those of figs. 4*a* and 6*b* where the rates of excretion of both sodium and potassium were low, carotid occlusion produced an increase of about 0·004 m.equiv/min. in the potassium excretion, about the same size as the increase in the excretion of sodium. On the other hand, when the rate of excretion of sodium was higher as in figs. 4*b*, 6*a* and *c*, carotid occlusion still produced the same small increase of about 0·004 m.equiv/min. in the potassium excretion, although there was now a much larger increase in the rate of excretion of sodium. In experiments like those of figs. 4*b* and 6*a* the increase in urine volume produced by carotid occlusion was accompanied by a fall in the urinary *concentration* of potassium. Thus, there were many similarities between the effect on potassium excretion of carotid occlusion and of the ingestion of 0·9 per cent sodium chloride.

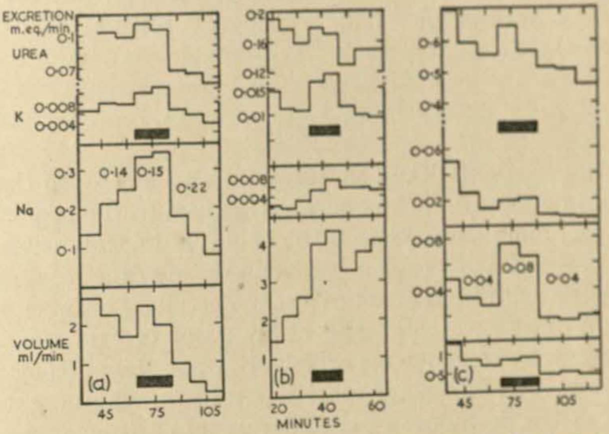

FIG. 6.—"Black Sue", weight 22 kg. The effect of occlusion of both carotid arteries (*a*) following priming doses of 0·9 per cent sodium chloride on the previous day and 350 ml. of 0·9 per cent sodium chloride at zero time, (*b*) with no priming doses of saline but 400 ml. of water by stomach tube at zero time and (*c*) with no priming doses but 100 ml. of 10 per cent urea by stomach tube at zero time. Plotting as in fig. 4, with the addition of rate of excretion of urea (m.equiv/min.) in the top frame.

Urea Excretion.—In five experiments on two dogs carotid occlusion was applied during the diuresis provoked by the administration of urea. In the example of fig. 6*c* occlusion was applied when the volume of urine and the rate of excretion of urea were falling following a test dose of 10 g. urea in 100 ml. of water; the time course of such a urea diuresis was described by O'Connor [1950]. In fig. 6*c* carotid occlusion had only a small effect on the rate of excretion of urea or the urine volume, although there was an obvious increase in the rate of excretion of sodium. In other experiments with or without administered doses of urea (*e.g.* fig. 6*a* and *b*) carotid occlusion never increased the rate of excretion of urea by more than 30 per cent, whereas the rate of excretion of sodium was always increased by 50–100 per cent.

Water Diuresis.—The effect of carotid occlusion during water diuresis is shown in fig. 6*b*; there was a definite increase in the volume of the urine and some increase also in the rate of excretion of sodium, potassium and urea. The nature and extent of the changes in fig. 6*b* were typical of ten similar experiments on three dogs.

DISCUSSION

In the previous paper [O'Connor, 1955 *b*] it was concluded that neither the renal nerves nor any known hormone was essential to the production of rates of excretion of sodium greater than 0·15 m.equiv/min., following the administration of saline to animals previously given priming doses of sodium chloride. In this paper sufficient differences have been described between the responses to the ingestion of saline at high and at low rates of excretion of sodium to make it necessary to consider separately the possibility that intermediary factors may be involved in changes at low rates of sodium excretion. The renal nerves and the hormones of the suprarenal cortex, suprarenal medulla and the neurohypophysis are four possible intermediary factors which must be considered.

The renal nerves do not appear to be essential in the production of changes in sodium excretion at any level since Berne [1952], Surtshin, Mueller and White [1952] and Surtshin and Hoeltzenbein [1954] have shown that in conscious dogs, sodium excretion by one denervated and one normal kidney remained equal at rates of excretion of sodium from 0·0005 to 0·3 m.equiv/min./kidney.

It does not appear possible to explain the increased excretion of sodium described in this paper as due to decreased production of salt-retaining hormone of the suprarenal cortex, since increased excretion of sodium was accompanied by increased excretion of potassium: in adrenalectomized dogs, where the action of desoxycortone or aldosterone is most clearly seen, injection of steroid causes decreased excretion of sodium and increased excretion of potassium; withdrawal of steroid therapy has the opposite effects and the effects of an injection of steroid appear and disappear much more slowly than the changes described in this paper [*e.g.* Garrod, Davies and Cahill, 1955; Liddle, Cornfield, Casper and Bartter, 1955].

Infusion of adrenaline reduces excretion of both sodium and potassium and indeed in many ways the effect on renal function of ending an infusion resembles the effect of ingestion of saline [O'Connor, 1958 *b*]. The effect of adrenaline is, however, very small compared to the effect of the ingestion of saline and is only produced by rates of infusion of adrenaline which have large effects on the heart rate. Also it is difficult to see by what mechanism ingestion of 100–400 ml. of saline would decrease the activity of the adrenal medulla. Similarities between the effect of adrenaline and ingestion of saline on renal function are more likely to reflect similar modes of action on the kidney than to indicate that change in rate of release of adrenaline is an intermediary factor determining the renal response to the ingestion of saline.

The influence of the neurohypophysis on slow rates of excretion of sodium requires further investigation. The hormones of the neurohypophysis play no obvious part in determining high rates of sodium [O'Connor, 1950], but observations of Brooks and Pickford [1957] suggest that both vasopressin and oxytocin have more effect at the low rates of sodium excretion now under consideration. No mechanism has, however, yet been described by which the ingestion of 250 ml. of 0·9 per cent sodium chloride would be expected to cause the release of neurohypophyseal hormones. The sodium concentration of the plasma, and so its osmotic pressure, is not changed by ingestion of saline and Verney [1947] has shown that it is this factor which is most important in determining the release of hormones from the neurohypophysis. O'Connor [1950] found no increase in the antidiuretic activity of urine after the administration of 350 ml. of 0·9 per cent sodium chloride by stomach tube.

In the absence of any convincing evidence that the response to ingested saline at low, as well as at high, rates of sodium excretion involves an intermediary action on the kidneys by either the renal nerves or hormones, it must be concluded that the changes after saline are due to the action on the kidney of some change in the composition of the circulating blood. A change in the blood consistently produced by ingestion of saline is dilution of the plasma proteins, here recorded as a fall in the solid content of the plasma and related in fig. 3 to the changes in the rate of excretion of sodium. The most likely interpretation of the results of this and the preceding paper [O'Connor, 1955 *b*] is that at all levels of excretion of sodium from 0·001 to 0·5 m.equiv/min. the increased excretion of sodium which follows ingestion of isotonic saline is due to the direct effect on the kidney of dilution of the plasma proteins.

This explanation receives support from the description in this and an earlier paper [O'Connor, 1955 *b*] of the renal effects of occlusion of both carotid arteries and the description in a later paper [O'Connor, 1958 *b*] of the effects of the infusion of adrenaline. O'Connor [1955 *b*] showed that the renal effects of carotid occlusion were not mediated by the renal nerves or by any known hormone and so were attributed to the direct effect on the kidney of increased arterial blood pressure. In this paper sufficient similarity has been found between the renal effects of the ingestion of saline and of carotid occlusion to suggest that each acts on the kidney in a similar way. Another circumstance with many points of similarity is the effect on renal function of the infusion of adrenaline [O'Connor, 1958 *b*]. The decreases in the excretion of sodium, potassium and urea and volume of the urine produced by infusion of adrenaline have characteristics similar to the increases reported in this paper on the ingestion of saline or occlusion of the carotid arteries. Ingestion of saline, carotid occlusion, and adrenaline may each be expected to alter glomerular function by dilution of the plasma proteins, by increased arterial blood pressure or by renal vasoconstriction. Thus, change in glomerular function is a mechanism common to these three circumstances which would explain the similarities of their effects on urine flow and composition.

Inulin and creatinine clearance is the measurement commonly used to assess changes in glomerular function, and O'Connor [1955 *b*] quoted the results of Wesson, Anslow, Raisz, Bolomey and Ladd [1950] to estimate the magnitude of the changes in glomerular filtration rate produced by the ingestion of saline. From the experiments of Wesson *et al.* [1950] in which very large doses of saline were infused in dogs, a fall of 0·1 g./100 g. in plasma solids would be expected to produce an increase of 1 ml./min. in glomerular filtration rate. Thus, in fig. 3*b* increase in sodium excretion from 0·002 to 0·011 m.equiv/min. was associated with a fall of 0·4 g./100 g. in the plasma solids and so should be accompanied by an increase of glomerular filtration rate from say 45 to 49 ml./min. Inaccuracies in the measurement of inulin or creatinine clearance are such that a change of this order could not be established by direct measurement under the existing conditions of a changing and very low rate of urine flow. Occlusion of the carotid arteries has been found (p. 377) to produce increases in sodium excretion equal to those due to a fall of 0·12–0·2 g./100 g. in the plasma solids and so need involve an increase of less than 2 ml./min. in glomerular filtration rate: in three experiments on carotid occlusion Sellwood and Verney [1955] did in fact find an increase of this order (5 per cent) in the creatinine clearance. The decrease in sodium excretion produced by adrenaline was such as to suggest that it would be associated with a fall of about 1·5 ml./min. in glomerular filtration rate [O'Connor, 1958 *b*]. The published data concerning inulin or creatinine clearances during the action of adrenaline is reviewed by O'Connor [1958 *b*], with the conclusion that it favours rather than excludes the possibility that a decrease of 4 per cent is produced by doses of adrenaline which decrease the excretion of sodium by about 50 per cent.

In the preceding paragraph the common features in the renal effects of the ingestion of saline, carotid occlusion and infusion of adrenaline are thus regarded as the effects on urine flow and composition of small changes in glomerular filtration. Reduction in glomerular filtration has been produced in conscious dogs by partial obstruction of the renal artery by Mueller, Surtshin, Carlin and White [1951], Leaf, Kerr, Wong and Chatillon [1954] and Berliner and Davidson [1957]. Usually, however, the obstruction has been such as to produce far larger reductions of glomerular filtration than those postulated in the previous paragraph, or the occlusion has been produced during diuresis produced by water or mannitol, so that only limited comparison can be made with the effects of saline, carotid occlusion or adrenaline described in this paper. However, the findings of Mueller *et al.* [1951] and Berliner and Davidson [1957] showed that reduction in sodium excretion was the most obvious effect of partial obstruction of the renal artery, and Berliner and Davidson [1957] found that potassium excretion was also reduced. Urine flow in water diuresis was also reduced by obstruction of the renal artery. One set of observations in the paper of Mueller *et al.* [1951] is of particular interest. In this group, occlusion of one renal artery reduced sodium excretion by that kidney from 0·005 to 0·003 m.equiv/min.; the data indicated a fall in glomerular filtration rate of about 1 ml./min., and although this was too small to be regarded as significant by the authors, it would be large enough to explain the change in sodium excretion in accordance with the considerations set out in the preceding paragraph.

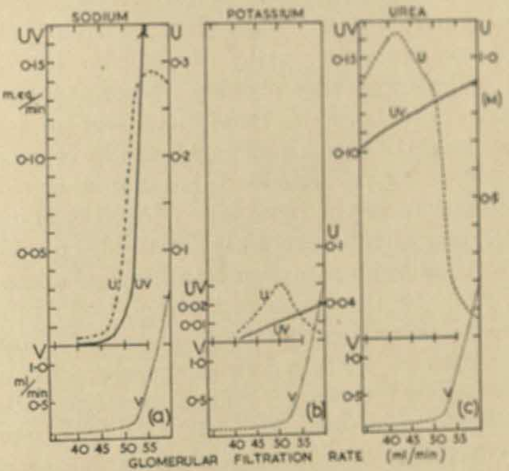

Fig. 7.—The postulated effects in dogs of changes in glomerular filtration on the urinary excretion of (*a*) sodium, (*b*) potassium and (*c*) urea. In each diagram arbitrary values for glomerular filtration rate (ml./min.) are plotted as abscissæ and the volume of the urine (ml./min.) is plotted as the dotted line V in the lower frame. In the upper frames the continuous line UV shows the rate of excretion of the solute (m.equiv/min., scale to left), and the broken line U its urinary concentration (Molar, scale to the right). For further explanation see text.

The effects of occlusion of the renal artery, ingestion of saline, carotid occlusion and infusion of adrenaline have been combined to give fig. 7, which is an attempt to summarize the effects on urine flow and composition apparently produced in dogs by sudden changes in glomerular filtration without changes in other factors affecting the kidney. The effects may form three different pictures according to the level of glomerular filtration at the time the sudden change is produced:

(1) At normal filtration rate, arbitrarily placed in fig. 7 as 45 ml./min. on the abscissa scale, the volume of the urine is represented in the lower frame of each figure as 0·12 ml./min.; in fig. 7*a* sodium excretion and concentration are shown in the upper frame as

0·002 m.equiv/min. and 0·015 M respectively; in fig. 7*b* potassium excretion and concentration as 0·004 m.equiv/min. and 0·03 M and in fig. 7*c* urea excretion and concentration as 0·12 m.equiv/min. and 1·0 M. These are values found in this paper when no doses of saline had been given. At this level an increase of glomerular filtration rate to 50 ml./min., as is presumed to occur on carotid occlusion or ingestion of saline, causes only a small change in the volume of the urine. Sodium excretion (fig. 7*a*) increases from 0·002 to 0·015 m.equiv/min. due mainly to the increase in sodium concentration to 0·1 M; potassium excretion and concentration increases also but to a smaller extent (fig. 7*b*). The excretion of urea (fig. 7*c*) increases by about 5–10 per cent with a small fall in its urinary concentration. Small decreases in glomerular filtration rate, from levels of about 45–50 ml./min. have corresponding effects as exemplified by the effects of adrenaline and by the effects of partial occlusions of the renal artery [Mueller *et al.*, 1951].

(2) When glomerular filtration rate has already been raised by 15 per cent to about 52 ml./min. a further small increase has different results, as exemplified by the effects of the ingestion of saline or of carotid occlusion following priming doses of saline. Now an increase of 5 per cent (to 55 ml./min.) in glomerular filtration increases the volume of the urine to 0·75 ml./min.; the rate of excretion of sodium (fig. 7*a*) increases from 0·03 to 0·18 m.equiv/min. with the sodium concentration of the urine remaining at about 0·25–0·3 M. Potassium and urea excretion are both increased also, but the urinary concentration of these substances falls owing to the increased volume of the urine. Decreased glomerular filtration as exemplified by the effects of adrenaline has the opposite effects. This picture holds at increasing glomerular filtration rates to give sodium excretion of 1 m.equiv/min. with glomerular filtration rates of 60–70 ml./min. as in the experiments of Wesson *et al.* [1950]. At such high rates of sodium excretion the concentration of sodium in the urine tends to fall towards 0·1 M.

(3) Reduction of glomerular filtration to about 50 per cent of the normal value is exemplified particularly in the results of Leaf *et al.* [1954] on obstruction of the renal artery and part of fig. 7*c* is based on their results. The excretion of urea falls approximately in proportion to the fall in glomerular filtration rate; urea excretion decreases mainly owing to a fall in the urinary concentration with little change in the volume of the urine.

Although reduction of glomerular filtration to low levels may limit the excretion of urea, within the normal physiological range the predominant effect of a change in the glomerular filtration rate appears to be a change in the rate of excretion of sodium. To this extent the relationship in fig. 3 between excretion of sodium and dilution of the plasma proteins is a specific and sensitive response by which the sodium content of the body is related to excretion. The importance of this relationship in the maintenance of normal sodium balance during high sodium intakes has been suggested in a previous paper [O'Connor, 1955 *b*] and its importance at lower intakes of sodium is being studied by work now in progress.

ACKNOWLEDGMENTS

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THE EFFECT ON THE VOLUME AND COMPOSITION OF THE URINE OF THE INFUSION OF ADRENALINE AND NORADRENALINE.

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The most prominent renal effect of the infusion of adrenaline in conscious dogs was a decrease in the rate of excretion of sodium. This required the infusion of adrenaline at 0·1–0·2 μg./kg./min. and was always accompanied by an increase in heart rate. A similar effect could be produced by the infusion of noradrenaline, but in larger doses (0·2–0·4 μg./kg./min.), and the effect was always accompanied by slowing of the heart and increased blood pressure.

The nature of the urinary changes depended on the rate of excretion of sodium at the time of the infusion. If the rate was less than 0·03 m.equiv/min., decreased excretion of sodium due to adrenaline was the result largely of a decrease in the sodium concentration in the urine, with only a small fall in the volume of the urine; at higher rates, the decrease in sodium excretion was produced by decreased volume of the urine without decrease in urinary sodium concentration. Adrenaline produced a small fall in the rate of excretion of potassium, but very little change in the excretion of urea. Urinary volume was decreased when adrenaline was infused during water diuresis.

Comparison was made between adrenaline and the ingestion of 0·9 per cent sodium chloride as agents altering the rate of excretion of sodium; the effect of adrenaline (0·1–0·2 μg./kg./min.) would be counteracted by a fall of 0·15 g./100 g. in the plasma solids produced by the ingestion of saline.

It is pointed out in discussion that the renal effects of adrenaline may be satisfactorily accounted for as due to a fall of about 3 per cent in glomerular filtration rate.

INTRODUCTION

The literature on the renal effects of adrenaline has been reviewed by Pickford [1952]. In most instances the effects have been produced only by large doses of the drug and so are unlikely to be of importance in the normal control of renal function. Several authors [for references see Blake, 1955] have emphasized that the most prominent action of adrenaline on the kidney is to reduce the rate of excretion of sodium. This effect is produced consistently and by small rates of infusion of adrenaline, and so has been further investigated as possibly indicating one factor involved in the normal control of sodium excretion.

In this paper the effects of adrenaline have been compared with the effects of ingestion of 0·9 per cent sodium chloride and of occlusion of the carotid arteries; these also are particularly effective in causing changes in the rate of excretion of sodium [O'Connor, 1955, 1958 *a*]. In addition a comparison has been made between the dose of adrenaline needed to reduce sodium excretion and that which produced tachycardia [Holgate and O'Connor, 1958]. Some effects of noradrenaline are also described. A preliminary account has already been published [O'Connor, 1958 *b*].

METHODS

The effects of adrenaline and noradrenaline on urine flow and composition were investigated during experiments in which blood pressure and heart rate were also recorded, with the results described by Holgate and O'Connor [1958]. The experiments were performed on three trained bitches ("Floss", "Skewbald" and "Black Sue"), which were also used for the experiments described by O'Connor [1958 *a*]. Operative preparations of the animals, details of the procedures and the analytical methods have already been described [Holgate and O'Connor, 1958 and O'Connor, 1958 *a*].

On the occasion of each individual experiment on the effect of adrenaline or noradrenaline, the animal was catheterized, and the chosen dose of urea, water or 0·9 per cent sodium chloride was given by stomach tube. The animal was then placed on its side on a warm table and lay quietly for the remainder of the experiment. Infusions of drugs were given through polythene tubing inserted into the malleolar vein and threaded forward so that the tip lay in the femoral or iliac vein. Infusion of 0·85 per cent saline at 0·26 ml./min. was established for at least half an hour before changing to a similar infusion containing adrenaline or noradrenaline. Urine was collected from the self-retaining catheter inserted at the beginning of the experiment. Where the urine flow was small (less than 0·5 ml./min.) the bladder was washed out at the end of each collection period by the procedure described by O'Connor [1958 *a*], but at higher flows the collecting tubing was merely drained by means of a T-piece close to the vulva.

RESULTS

The Effective Dose of Adrenaline and Noradrenaline.—As will be illustrated later in this paper, the effect of adrenaline was most clearly seen when the rate of excretion of sodium had been raised by the previous administration to the animal of 0·9 per cent sodium chloride. In the experiments of fig. 1 infusion of adrenaline was begun about 50 min. after the administration of 350 ml. of 0·9 per cent sodium chloride by stomach tube. The animal had also been given by stomach tube 200 ml. of saline 4 hr. previously and a dose of 300 ml. on the days preceding the experiment. The response to the administration of 0·9 per cent sodium chloride under these conditions has been described in previous papers [O'Connor, 1950, 1955]; in the examples of fig. 1, 50 min. after the test dose the rate of excretion of sodium had risen to 0·25 m.equiv/min. and in the absence of the infusion would have remained at this level or risen a little further in the next 20 min. However in fig. 1*a* and *b* the infusion of adrenaline at 0·073 and 0·17 μg./kg. body weight/min. caused the rate of excretion of sodium to fall to 0·18 and 0·08 m.equiv/min., about 75 and 30 per cent of the expected rate in the absence of any infusion. The numerals at the top of fig. 1 give the heart rate and the mean arterial blood pressure; in both experiments adrenaline caused a large increase in heart rate, as has been described by Holgate and O'Connor [1958].

Fig. 2 shows on the same animal and under the same conditions the effect of the infusion of noradrenaline at 0·12 and 0·4 μg./kg./min. and again the rate of excretion of sodium was reduced by the infusions. The changes in heart rate and mean arterial blood pressure shown at the top of the fig. 2 are typical of the effects of noradrenaline on the circulation [Holgate and

O'Connor, 1958]; there was a slowing of the heart rate and, with the higher dose, an increase in arterial blood pressure.

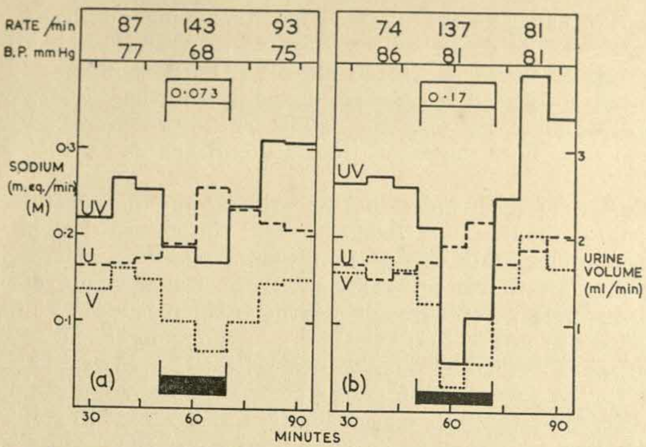

FIG. 1.—"Skewbald", 15 kg. The effect on sodium excretion of the infusion of adrenaline at (*a*) 0·073 and (*b*) 0·17 μg./kg./min., during the period marked by the rectangles. Abscissæ; time in min. Ordinates: continuous line (UV), rate of excretion of sodium, m.equiv/min., scale on the left; broken line (U), urinary sodium concentration, molar, scale on left; dotted line (V), rate of urine flow, ml./min., scale on right. Figures at the top give the mean arterial blood pressure (mm. Hg) and the pulse rate (per min.). Doses of 0·9 per cent sodium chloride were given by stomach tube; 300 ml. on the previous day, 200 ml. 4 hr. before the experiment and 350 ml. at zero time of the graph.

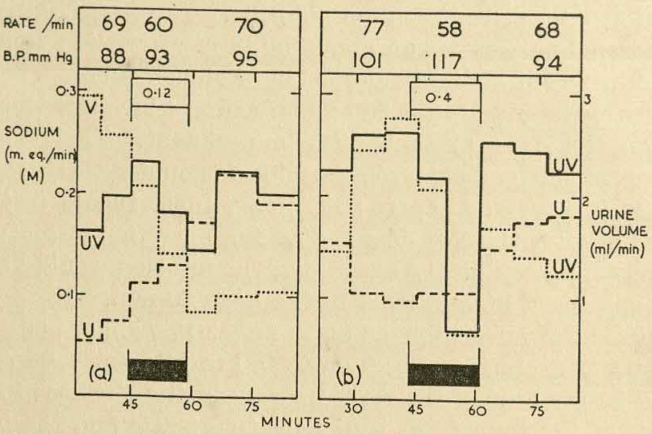

FIG. 2.—"Skewbald", 15 kg. The effect on sodium excretion of infusion of noradrenaline at (*a*) 0·12 and (*b*) 0·4 μg./kg./min. Plotting and experimental conditions as in fig. 1.

The effect of different rates of infusion of adrenaline was investigated under the conditions of fig. 1 in twenty-four experiments on three dogs, with the results shown in fig. 3*a*. Infusion at 0·07 μg./kg./min. or more always produced a decrease in sodium excretion and infusion at about 0·15 μg./kg./min.

was required to reduce sodium excretion to about half of the value expected in the absence of an infusion. Holgate and O'Connor [1958, fig. 3*a*] plotted in the same way the increase in heart rate and the small changes in blood pressure produced by adrenaline in these same experiments; comparison of the two figures illustrates the fact that when the dose of adrenaline was large enough to decrease the rate of excretion of sodium, there was always a large increase in heart rate, as in the examples of fig. 1*a* and *b*.

The effect of noradrenaline on the excretion of sodium was less than that of an equal infusion of adrenaline; fig. 3*b* shows its effect on sodium excretion in fifteen experiments on the three dogs. Infusion of 0·15 μg./kg./min. was needed to produce consistently recognizable decreases in sodium excretion. Infusions of noradrenaline which produced a decrease in sodium excretion were always large enough to produce the characteristic effects of noradrenaline on the circulation, namely slowing of the heart rate and an increase in arterial blood pressure [Holgate and O'Connor, 1958, fig. 3*b*].

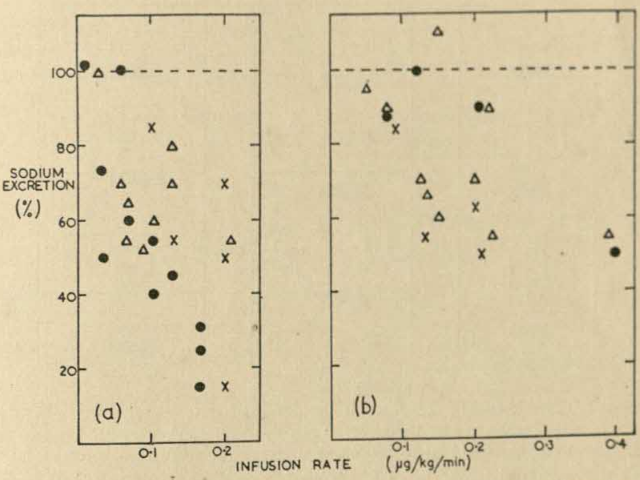

FIG. 3.—The effect on sodium excretion of different rates of infusion of (*a*) adrenaline and (*b*) noradrenaline. Abscissæ; rate of infusion (μg./kg./min.). Ordinates, rate of excretion of sodium in experiments like those of figs. 1 and 2: excretion during the infusion is expressed as a percentage of the mean of the rates before and after the infusion. Experiments on "Skewbald" shown by circles, on "Floss" by triangles and on "Black Sue" by crosses.

The Effect of Adrenaline on Sodium Excretion.—In the experiments of fig. 1 and other examples in figs. 4*a* and 6*a*, infusion of adrenaline decreased the excretion of sodium in the same way; in each case the concentration of sodium in the urine was not decreased by the infusion, but the decreased rate of excretion of sodium was due to a fall in the rate of flow of the urine. The examples of figs. 1, 4*a* and 6*a* are typical of the effect of adrenaline when the rate of excretion of sodium had been raised by the previous administration of 0·9 per cent sodium chloride.

In fig. 4*c*, adrenaline was infused at 0·2 μg./kg./min. without any previous administration of sodium salts and so the rate of excretion of sodium was low (0·003 m.equiv/min.). It was difficult to recognize any effect of adrenaline on sodium excretion or urinary volume under these conditions. In six experiments on two animals adrenaline was infused at 0·1–0·2 μg./kg./min. when the rate of excretion of sodium was 0·003–0·006 m.equiv/min.; in each case, as in fig. 4*c*, any fall in sodium excretion was small, being of the order of only 0·001 m.equiv/min.

In the experiment of fig. 4*b*, 300 ml. of 0·9 per cent sodium chloride was given by stomach tube on the day before the experiment and so the rate of excretion of sodium was 0·01 m.equiv/min. when the infusion of adrenaline was given. During the infusion sodium excretion fell to 0·002 m.equiv/min.; the fall in the rate of excretion of sodium was due to a fall in the urinary sodium concentration from 0·11 to 0·025 Molar with only a small decrease in the rate of urine flow. There were six experiments on two dogs when adrenaline was infused at 0·1–0·2 μg./kg./min. with the rate of excretion of sodium 0·009–0·03 m.equiv/min.; sodium excretion was decreased by about 0·008 m.equiv/min. and in each case there was a fall in urinary sodium concentration with a somewhat smaller decrease in the rate of flow of urine.

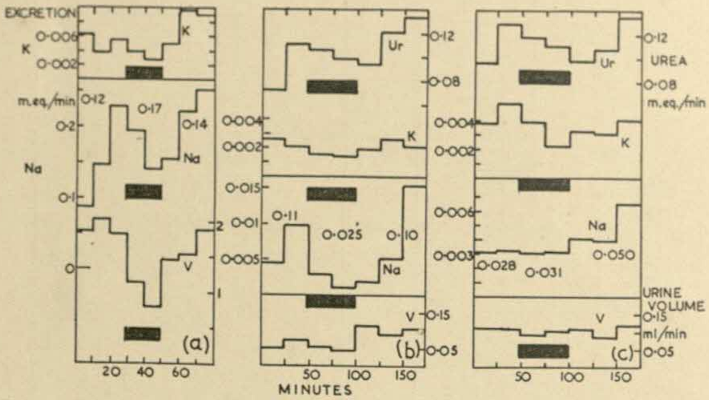

Fig. 4.—"Black Sue", 21 kg. The effect on the excretion of sodium, potassium and urea of the infusion of adrenaline. Abscissæ; time in min. Ordinates: from below upwards the urinary volume (ml./min.) and the rates of excretion of sodium, potassium and urea (m.equiv/min.). The figures on the curve for sodium give its urinary concentration (Molar). Infusion in (*a*) at 0·13 μg./kg./min. following priming doses and 350 ml. of 0·9 per cent sodium chloride at zero time, (*b*) at 0·2 μg./kg./min. following 300 ml. of saline on previous day and (*c*) at 0·2 μg./kg./min. with no doses of saline.

The decreased excretion of sodium produced by adrenaline thus had several features in common with the increase in sodium excretion produced by the ingestion of saline or occlusion of both carotid arteries [O'Connor, 1958 *a*]. In each case the effect on sodium excretion was much larger when the rate of excretion of sodium had been increased by the prior administration of 0·9 per cent sodium chloride; at high rates of excretion of sodium, the changes produced by the ingestion of saline, carotid occlusion or adrenaline were due to changes in the volume of the urine without change in the urinary sodium concentration; at low rates of excretion of sodium (below about 0·03 m.equiv/min.) changes in sodium excretion were largely due to changes in the urinary sodium concentration without great changes in the urinary volume.

The effect of adrenaline was, however, small in comparison with the effect of ingestion of 0·9 per cent sodium chloride, and the effect of these two agents is compared in fig. 5. In the previous paper [O'Connor, 1958 *a*] the rate of excretion of sodium was plotted against the plasma solid content following ingestion of saline to give a curve which is reproduced as the solid line in fig. 5. Each experiment in which adrenaline was infused is plotted in fig. 5 as follows, using the experiment of fig. 4*b* as an example. On this occasion adrenaline reduced the excretion of sodium from 0·011 to 0·002 m.equiv/min.; this was plotted as the closed circle in fig. 5 by reading from the continuous line that sodium excretion of 0·011 m.equiv/min. corresponded to a plasma solid content of 7·86 and the point showing the effect of adrenaline was then plotted as 7·86, 0·002 by assuming that the solid content of the plasma was not changed during the short period of the infusion. In fig. 5 the points from nine experiments on "Black Sue" all fell reasonably close to the dotted line drawn 1·5 divisions (0·15 g./100 g. plasma solids) to the right of the normal line. The effect of the adrenaline infusions was thus equal to the effect of a rise of 0·15 g./100 g. in plasma solids in experiments with "Black Sue", and was of similar magnitude in two other animals.

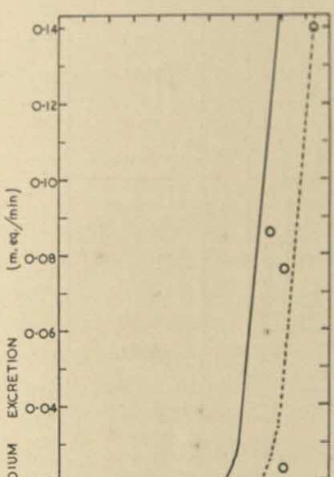

FIG. 5.—"Black Sue", 21 kg. Comparison of the effect of adrenaline on sodium excretion with that of the ingestion of 0·9 per cent sodium chloride. Abscissæ; plasma solid content (g./100 g.). Ordinates, rate of excretion of sodium (m.equiv/min.). The continuous curve is replotted from O'Connor [1958 *a*] and shows the relationship following the administration of saline in the absence of adrenaline: the circles and dotted line, plotted as described in the text, show the relationship during the infusion of adrenaline at 0·1–0·2 μg./kg./min.

The Effect of Adrenaline on Potassium Excretion.—In eighteen experiments on two dogs the urinary content of potassium as well as of sodium was observed during the infusion of adrenaline at 0·1–0·2 μg./kg./min. and in fifteen of them the rate of excretion of potassium was decreased during the infusion; clearly in fig. 6*a*, and also in figs. 6*b*, 4*a*, *b* and *c* the effect of adrenaline was to reduce potassium excretion. The existing rate of excretion of potassium was always low, being 0·002–0·008 m.equiv/min. in six experiments where previous doses of 0·9 per cent sodium chloride had not been given (*e.g.* fig. 4*c*); 0·001–0·007 in five experiments in which small doses of sodium chloride were given (*e.g.* fig. 4*b*); and 0·005–0·035 m.equiv/min. in seven experiments in which large doses of saline were given (*e.g.* fig. 4*a*). The average falls in potassium excretion produced by adrenaline in the three groups of experiments was 0·003, 0·002 and 0·007 m.equiv/min. Although the quantitative estimate can only be tentative, the decreased excretion of sodium produced by adrenaline was usually accompanied by a decreased excretion of potassium, never an increase, and the renal response to adrenaline had this feature also as a point of similarity when compared with the effects of the ingestion of saline or carotid occlusion [O'Connor, 1958 *a*].

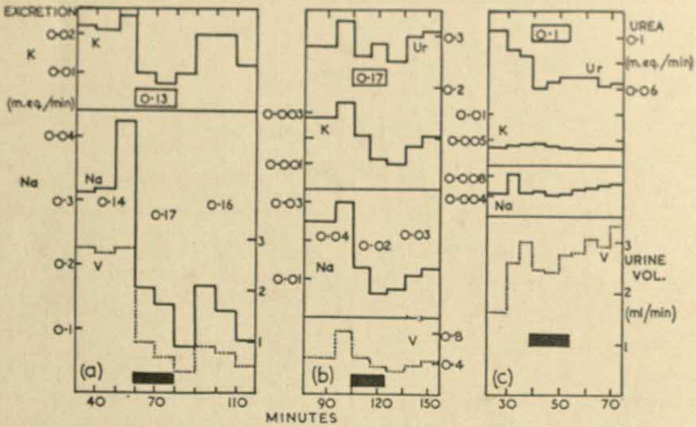

FIG. 6.—"Skewbald", 15 kg. The effects of the infusion of adrenaline on the excretion of sodium, potassium and urea. Plotting as in fig. 4. By stomach tube at zero time in (*a*) 350 ml. 0·9 per cent sodium chloride, in (*b*) 5 g. urea in 100 ml. water and in (*c*) 320 ml. water. Rates of infusion in (*a*) 0·13, (*b*) 0·17 and (*c*) 0·1 μg./kg./min.

The Effect of Adrenaline on the Excretion of Urea.—Whatever the existing rate of excretion of sodium, the rate of excretion of urea was usually diminished a little during an infusion of adrenaline, as in the examples of fig. 4*b* and *c*. Of fourteen experiments on two dogs, the rate of excretion of urea was decreased by adrenaline (0·1–0·2 μg./kg./min.) in ten, unchanged in two and increased in two; the mean fall in the fourteen experiments was 10 per cent. This fall was too small to be established with certainty against the random variations which frequently occurred between urea excretion in successive periods especially when the urine flow was low or changing rapidly. Fig. 6*b* shows an experiment in which 5 g. of urea in 100 ml. of water was given by stomach tube; urea excretion followed the time course described by O'Connor [1950] without any large effect of the infusion of adrenaline at 0·17 μg./kg./min. Fig. 6*b* is typical of three experiments in which the effect of adrenaline was tested after the administration of urea.

The Effect of Adrenaline Infused during Water Diuresis.—Infusion of adrenaline during the rising phase of water diuresis in fig. 6c produced some slowing of the diuresis, and this experiment was typical of five experiments with two dogs where adrenaline was infused at 0·1–0·2 μg./kg./min. during water diuresis. The rate of flow of urine in each experiment was at most halved. The time course of the inhibition in fig. 6c was typical in that the effect of the adrenaline disappeared over a period of about 20 min. after the end of the infusion. The effect of adrenaline on sodium excretion (fig. 1, 4) and on the heart rate [Holgate and O'Connor, 1958] were similarly recognizable for about 20 min. after the end of the infusion. Thus, in fig. 6c and similar experiments there was no clear evidence of a prolonged inhibition of water diuresis due to the liberation of neurohypophyseal hormone by adrenaline, as was described by Dearborn and Lasagna [1952] and Pickford and Watt [1951].

DISCUSSION

The most apparent effect on renal function of small doses of adrenaline infused in conscious dogs is decreased excretion of sodium. In my experiments infusion at 0·1–0·2 μg./kg./min. produced a decrease of about 50 per cent; similar decreases in sodium excretion were produced by Berne, Hoffman, Kagan and Levy [1952] with 1–2 μg./kg./min. and by Blake [1955] with 0·2–0·35 μg./kg./min.; while Kaplan, Fomon and Rapoport [1952] found that 0·1–1·4 μg./kg./min. decreased sodium excretion during the course of mannitol diuresis. The rate of infusion needed to slow sodium excretion was thus quite small, but even so it was enough to cause a large increase in the heart rate in the present work and apparently also in the experience of Blake [1955]. It seems unlikely, therefore, that changes of the rate of entry of adrenaline into the blood stream can be an important factor in determining the sodium balance of the body, since changes in the rate of excretion of sodium are not normally accompanied by changes in the heart rate.

The effect on sodium excretion of noradrenaline has been studied less than the effect of adrenaline. It has been shown (fig. 3) that noradrenaline had to be infused at about twice the dose of adrenaline in order to produce the same effect on sodium excretion, and a similar difference in dose was also required to produce their characteristic effects on pulse rate and arterial blood pressure [Holgate and O'Connor, 1958]. Kaplan *et al.* [1952], infusing the amines in conscious dogs at 0·1–1·4 μg./kg./min., found that adrenaline produced greater reduction in sodium excretion than did noradrenaline. Berne *et al.* [1952] found them equally active but at higher rates of infusion (1–2 μg./kg./min.). In anæsthetized dogs, adrenaline is more effective than noradrenaline in constricting the renal vessels [Burn and Hutcheon, 1940; Ahlquist, Taylor, Rawson and Sydow, 1954; Page and McGubbin, 1953]. The difference in the actions of the two amines on sodium excretion may not be solely due to a weaker action of noradrenaline on the kidneys. In the present experiments noradrenaline produced an increase of 10–20 mm. Hg in arterial blood pressure [Holgate and O'Connor, 1958] and this would be expected to cause an increase of about 20 per cent in the rate of excretion of sodium [O'Connor, 1955, 1958 *a*]. The effect of noradrenaline on sodium excretion may thus be the resultant of two opposing actions, namely the renal action of noradrenaline and its hypertensive action. Adrenaline had no effect on the blood pressure [Holgate and O'Connor, 1958].

Superficially, the fact that adrenaline reduced the excretion of sodium and potassium without greatly affecting the excretion of urea suggests that it has a specific effect on the handling of electrolytes by the renal tubules. However, as described in this paper, the renal action of adrenaline had many features similar to the effects in the same animals of ingestion of 0·9 per cent sodium chloride or occlusion of both carotid arteries. In previous papers [O'Connor, 1955, 1958 *a*] the effect of the ingestion of saline was attributed to increased glomerular filtration produced by dilution of the plasma protein, and the effect of carotid occlusion to the increased glomerular filtration produced by the raised arterial blood pressure. The similarities suggest that the effects of adrenaline are due to a fall in glomerular filtration produced by its vasoconstrictor action on the renal vessels. This explanation appears to be accepted by Blake [1955] as accounting for at any rate a large part of the renal effects of adrenaline.

Quantitatively, the effect of adrenaline was small in comparison with the increased excretion of sodium produced by the ingestion of saline; according to fig. 5 it was equal to the effect of a fall of 0·15 g./100 g. in plasma solids. From considerations based on the results of Wesson, Anslow, Raisz, Bolomey and Ladd [1950] and discussed by O'Connor [1955], a rise of 0·15 g./100 g. in plasma solids corresponds to a fall of 1·5 ml./min. in glomerular filtration rate, and so a change of glomerular filtration rate from 50 to 48·5 ml./min. is all that need be postulated to explain the renal actions of adrenaline described in this paper. Mannitol, inulin or creatinine clearances during the infusion of adrenaline in conscious dogs have been reported by several authors. Kaplan *et al.* [1952] found a reduction of 10 per cent or more; Berne *et al.* [1952] found a reduction of 15 per cent in denervated kidneys but no change in normal kidneys; Blake [1955] found a fall of 10 per cent in the first clearance period during the infusion and a change varying from +20 to −20 per cent in the second collection period. Thus, many of the results do in fact show a fall, the statistical significance of which cannot be assessed from the data provided. The data in fact favours and is totally inadequate to exclude, a fall of 3 per cent in glomerular filtration rate and so cannot contradict the explanation that the effect of adrenaline on the excretion of sodium is due to a small fall in glomerular filtration.

Some actions of adrenaline which have been described in the literature were not observed in my experiments. In the perfused kidney and in anæsthetized animals adrenaline may increase the flow of urine [Richards and Plant, 1922; Winton, 1931; Toth, 1937]. In a recent review, Pickford [1952] expressed the impression that increased flow of urine was more likely when small doses of adrenaline were injected or infused. I have never observed this in conscious dogs with rates of infusion as low as 0·01 μg./kg./min.

Dearborn and Lasagna [1952] produced a prolonged inhibition of water diuresis by large single injections of adrenaline and showed that this was due to the release of antidiuretic hormone from the neurohypophysis. Pickford and Watt [1951] found that infusion of adrenaline (0·47 μg./kg./min.) produced an inhibition outlasting the probable duration of the action of adrenaline, but such an effect was not observed in my experiments. O'Connor and Verney [1945] found that single injections of adrenaline could inhibit the release of antidiuretic hormone from the neurohypophysis during emotional stress. There has been no indication in the present experiments that infusion of adrenaline could block the normal release of antidiuretic hormone and so produce a transient diuresis.

Acknowledgment

I wish to thank Mr. J. Brook for his very patient technical assistance.

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THE EFFECT OF INTRAVENOUS INFUSION OF NORADRENALINE ON THE RESPIRATORY RESPONSE TO CARBON DIOXIDE IN MAN.

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Continuous intravenous infusion of 8–15 μg./min. noradrenaline in man with alveolar pO_2 held at about 140 mm. Hg usually increases the slope of the linear part of the relation between ventilation and alveolar pCO_2. It does not significantly alter the intercept of this line with the pCO_2 axis. The response after the beginning of an infusion of noradrenaline often takes 15–20 min. to reach a steady state.

Barcroft, Basnayake, Celander, Cobbold, Cunningham, Jukes and Young [1957] showed that the increase in pulmonary ventilation ($\dot{V}$, l./min., B.T.P.S.) associated with an intravenous infusion of noradrenaline in man persists if the reduction in the alveolar CO_2 is kept small by adding some CO_2 to the inspired air. Their results did not allow them to decide whether noradrenaline affected the slope (S) or the intercept (B) of the linear relation between $\dot{V}$ and the partial pressure of CO_2 in the alveolar air (pCO_2, mm. Hg), which may be expressed by the equation $\dot{V} = S(pCO_2 - B)$ [Lloyd, Jukes and Cunningham, 1958].

We have attempted to answer this question in the present work. In any one session the $\dot{V}$–pCO_2 relation has been observed during prolonged infusions of saline and noradrenaline; pooling of results from different days has been unnecessary; and the partial pressure of oxygen (pO_2) in the alveolar air has been kept at one level. The approach to the respiratory steady state after changes in the infusion or in the inspired gas mixture has been followed to completion, and the pattern of this approach has been investigated thoroughly in supplementary experiments.

Methods

The respiratory methods previously described [Cunningham, Cormack, O'Riordan, Jukes and Lloyd, 1957; Lloyd *et al.*, 1958] have been used; end-tidal air was drawn continuously through the flowbridge analyzer for estimation of pCO_2 and towards the end of each steady state into a sampling tube. Analysis in Haldane's apparatus gave the pO_2 and a value of pCO_2 for calibration of the flowbridge analyzer. The subjects sat back in a dental chair, one arm rest of which had been modified to support a supinated forearm. All apparatus except the infusion tubing and the respiratory valve with its immediate appendages were outside the field of vision of the subjects. They were post-absorptive healthy male undergraduates whose ages and body measurements are shown in Table I. They read books or papers for most of the time and were unaware that they had received anything other than saline and mixtures of air and CO_2.

Saline was infused according to the technique of Barcroft *et al.* [1957] at 2·7 ml./min., L-noradrenaline made up in a proprietary medium ("Levophed", Bayer Products Ltd.) being added when required to give a dosage of 8–15 µg./min. The proprietary medium without noradrenaline ("Pseudo-levophed") had no detectable respiratory effects [*cf.* Barcroft *et al.*, 1957]. The doses were chosen to produce a clear response without causing disturbing symptoms. On the few occasions when a troublesome headache occurred the infusion was stopped and the observations discarded.

TABLE I.—STATISTICS OF THE REDUCED MAJOR AXES * FOR $\dot{V}$ AND pCO_2 DURING INFUSIONS OF SALINE AND NORADRENALINE

Subjects	Experiment	Dose µg./min.	Intercept (B) ±s.e., mm. Hg CO_2		Slope (S) ±s.e., l./min. B.T.P.S./mm. Hg CO_2		Significance of difference between slopes
			Saline	Noradrenaline	Saline	Noradrenaline	
26 22 years 71 kg. 184 cm.	26, 2	10	39·5 ±0·6	40·1 ±0·3	3·84 ±0·27	5·90 ±0·24	$P < 0{\cdot}01$
	26, 3	10	40·9 ±0·3	40·4 ±0·5	4·31 ±0·14	5·77 ±0·42	$P < 0{\cdot}01$
	26, 4	10	40·0 ±0·5	39·6 ±0·5	4·83 ±0·34	5·91 ±0·58	$P > 0{\cdot}2$
	26, 5	10	38·7 ±0·4	38·6 ±0·7	4·09 ±0·18	5·72 ±0·49	$P < 0{\cdot}02$
27 22 years 82 kg. 183 cm.	27, 2	8	37·6 ±0·9	37·2 ±0·7	3·00 ±0·19	3·64 ±0·21	$P < 0{\cdot}07$
	27, 3	12	37·2 ±1·1	37·1 ±0·6	2·84 ±0·20	3·62 ±0·14	$P < 0{\cdot}02$
	27, 4	12	41·4 ±0·7	41·1 ±0·3	3·57 ±0·23	3·81 ±0·12	$P > 0{\cdot}4$
28 23 years 82 kg. 193 cm.	28, 1	10	39·6 ±0·3	38·8 ±1·9	4·51 ±0·15	6·04 ±1·20	$P > 0{\cdot}3$
	28, 3	15	38·7 ±0·4	38·3 ±0·2	5·13 ±0·24	5·78 ±0·10	$P < 0{\cdot}05$
	28, 4	15	37·3 ±0·4	36·5 ±0·3	4·73 ±0·22	5·89 ±0·19	$P < 0{\cdot}01$
29 22 years 78 kg. 179 cm.	29, 1	10	37·9 ±0·6	38·2 ±0·6	4·35 ±0·27	4·36 ±0·35	$P > 0{\cdot}9$
	29, 2	10	37·1 ±0·6	36·8	3·61 ±0·24	3·77	. .
	29, 3	10	37·9 ±0·2	37·4 ±0·6	4·38 ±0·06	3·84 ±0·20	$P > 0{\cdot}1$
31 21 years 80 kg. 179 cm.	31, 1	10	36·0 ±0·5	35·0 ±0·6	3·32 ±0·16	4·03 ±0·26	$P < 0{\cdot}05$
	31, 2	10	35·6 ±0·3	34·6 ±0·4	3·33 ±0·10	3·82 ±0·18	$P < 0{\cdot}05$
32 21 years 69 kg. 183 cm.	32, 1	10	37·3 ±0·8	38·4 ±0·1	4·01 ±0·38	5·83 ±0·06	$P < 0{\cdot}01$
	32, 2	10	39·2 ±0·3	38·7 ±0·6	6·69 ±0·36	6·09 ±0·58	$P > 0{\cdot}4$
	32, 3	10	40·6 ±0·2	40·3 ±0·3	6·02 ±0·16	6·42 ±0·37	$P > 0{\cdot}4$

* The major axes in normalized co-ordinates (*cf.* Kendall, 1957) give an almost identical set of values for B and S when the ratio of the probable errors in $\dot{V}$ to those in pCO_2 is assessed as 6:1

Our attention has been confined to the middle range of the $\dot{V}$–pCO_2 relation (fig. 1). A set of about five inspired gas mixtures was given, usually in order of ascending CO_2 content, during a single continuous infusion of noradrenaline lasting about an hour and a half, and a similar set was given during a saline infusion. The order of the infusions was varied and in two experiments the noradrenaline line was assessed twice. The gas mixtures were calculated to give an effectively constant alveolar pO_2.

In all but the earliest experiments the approach to the steady state was watched continuously in terms of $\dot{V}$ and pCO_2, and when drift in the response appeared to have ceased the steady-rate readings were taken over a period usually of 5 min., and finally averaged to give the values plotted in fig. 1. In certain subjects persistent reciprocal oscillation in $\dot{V}$ and pCO_2 with a periodicity of some 5 min. occurred; in such cases mean values over suitably prolonged intervals were taken to represent the steady state. In a supplementary series of experiments the response to a change from saline to noradrenaline was observed for between 30 and 60 min. Ten sets of observations were made at high and twelve at relatively low levels of $\dot{V}$, pCO_2 being held as constant as possible by adjustment of the inspired CO_2.

RESULTS

The Steady State.—$\dot{V}$ is plotted against alveolar pCO_2 for each of the main experiments in fig. 1.

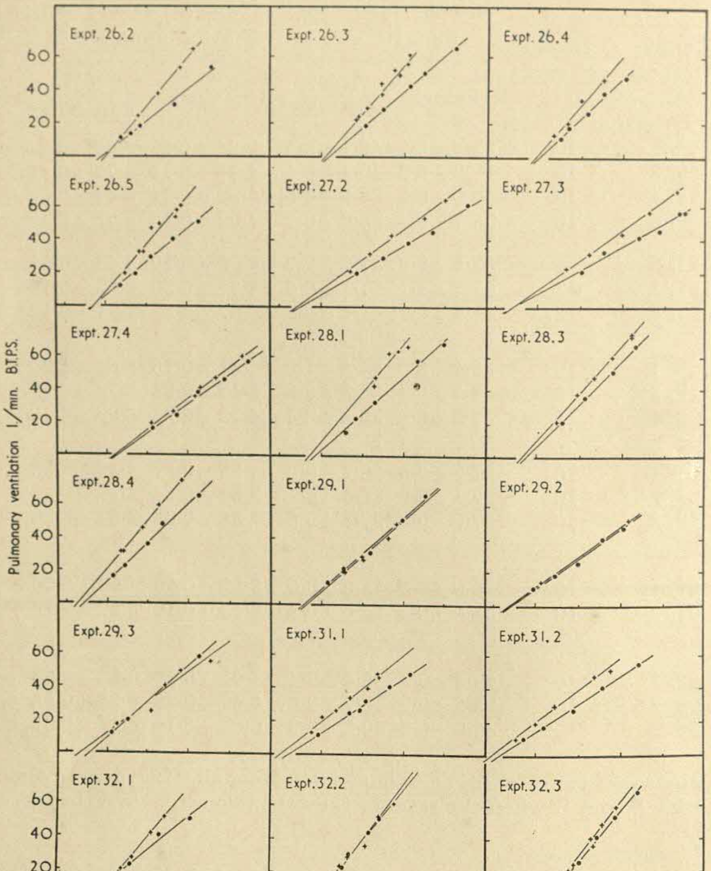

FIG. 1.—The effect of noradrenaline on the steady-state relation between pulmonary ventilation and alveolar pCO_2 for all eighteen experiments. The lines are the reduced major axes and have been produced to their intersections (B) with the pCO_2 axis: ● saline infusion; + noradrenaline infusion.

The mean alveolar pO_2 was 139·2 (s.d. 5·0) mm. for saline and 138·9 (s.d. 5·5) mm. for noradrenaline infusions. The lines drawn in fig. 1 are the reduced major axes of Kermack and Haldane [1950], and their slopes (S) and intercepts (B) on the pCO_2 axis are given in Table I.

In the three experiments on subject 29 noradrenaline infusion had no appreciable effect on either S or B, although the dose was sufficient to slow the heart rate. In nine out of fifteen experiments on the other five subjects noradrenaline was associated with a significant though small increase in S;

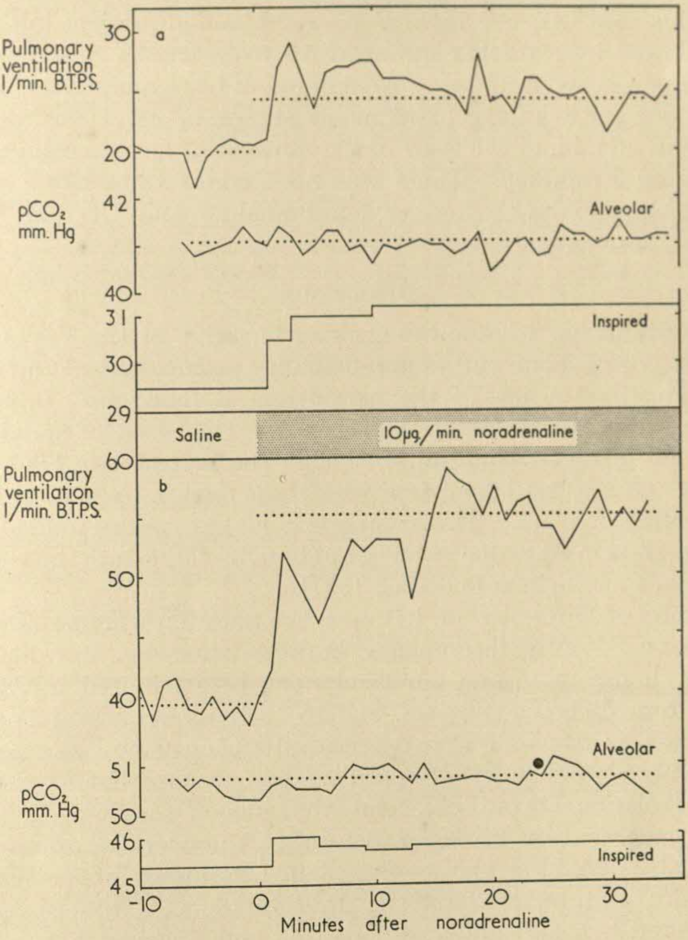

FIG. 2.—Typical examples of the approach to the steady state after changing from saline to noradrenaline infusion when alveolar pCO_2 was held as constant as possible at the low (*a*) and the high (*b*) levels; ● pCO_2 measured in Haldane's apparatus. The dotted horizontal lines represent the mean values in the steady states.

the day-to-day variation in S was smaller for noradrenaline than for saline. B usually fell by a fraction of a millimetre when noradrenaline was infused but no change in any experiment was statistically significant.

The Approach to the Steady State.—After a change of the inspired gas mixture during the course of an infusion the approach to the new steady state was similar whether saline or noradrenaline was being infused. After a change from saline to noradrenaline the amplitude of the reciprocal oscillation of $\dot{V}$ and

pCO_2 often increased temporarily. When the inspired CO_2 was low $\dot{V}$ usually increased abruptly by about 10 l./min., overshooting the steady-state value and then approaching it from above. This happened in eight out of the twelve supplementary experiments at low inspired CO_2 in which alveolar pCO_2 was kept nearly constant during and after the change of infusion, as in fig. 2*a*. When the inspired CO_2 was high, $\dot{V}$ showed a similar abrupt initial increase usually followed by a further upward drift towards the steady-state value, which was approached from below in eight out of ten experiments, as in fig. 2*b*. In all but one (28.3) of the experiments shown in fig. 1 the response was steady within 20 min. of the start of the infusion of noradrenaline.

Respiratory Frequency.—There was no obvious difference between the effects of saline and CO_2 and of noradrenaline and CO_2 on respiratory frequency.

DISCUSSION

The results in fig. 1 show the striking linearity of the $\dot{V}$–pCO_2 relation during infusions of saline and of noradrenaline at constant pO_2 in the steady state, and justify the use of the parameters B (horizontal intercept) and S (slope) in summarizing the data (Table I). The effect of noradrenaline is seen to be on S, rather than on B, though the increases in S never exceed 60 per cent and are small compared with those seen in hypoxia, when S may increase tenfold or more. This comparison makes it very unlikely that the hypoxic increases in S are due to release of noradrenaline into the venous blood [*cf.* Cormack, Cunningham and Gee, 1957].

The results of Barcroft *et al.* left open the question whether noradrenaline affected B or S. Any discrepancy between their fig. 2, which perhaps indicates a change in B, and our results can be attributed to their pooling of results from different subjects, and to their experimental procedure, in which single $\dot{V}$–pCO_2 points were determined during short-lasting infusions of saline and noradrenaline in alternation. In our experiments the whole of the $\dot{V}$–pCO_2 relation was outlined before the infusion was changed, a procedure previously suggested by Professor Barcroft. Our work on the approach to the steady state after the change from saline to noradrenaline suggests that if readings are taken before the steady state is attained $\dot{V}$ will be overestimated at low inspired CO_2 and underestimated at high inspired CO_2. This gives a false picture in which S appears to be left constant and B to be diminished by noradrenaline. Replotting the observations of Barcroft *et al.* for 8 and 13 min. (their fig. 1) separately bears this out, and allows us to reaffirm our conclusion that noradrenaline usually increases, albeit rather slightly, the slope of the $\dot{V}$–pCO_2 relation. Sensitivity, rather than threshold, is affected.

ACKNOWLEDGMENTS

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SURGICAL AND CHEMICAL TECHNIQUES FOR *IN VIVO* STUDIES OF THE METABOLISM OF THE INTESTINAL MICROFLORA OF DOMESTIC FOWLS.

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Two techniques have been developed so that the microbiology of the intestinal canal can be studied. First, a method of cæcectomizing day-old chicks is described so that the intestinal flora can develop in the absence of a cæcal one. Droppings collected from the birds are thus a convenient method of sampling this intestinal flora. Such birds appeared normal up to 12 weeks of age, apart from the absence of cæca.

So that the metabolism of the cæcal flora can be repeatedly studied in the same bird, a fistulation technique has been used. A chemical technique has been developed, based upon the hypothesis that ecological changes within the cæcum will be reflected in changes in the metabolic activity of the microflora. So that these changes may be observed *in situ,* a qualitative and quantitative assessment of the rate of gaseous production has been chosen as an index of metabolic activity.

INTRODUCTION

IN chicken the greatest numbers of micro-organisms occur in the cæca [Barnes and Shrimpton, 1957], which are paired blind tubes arising at the junction of the small and large intestine, and so the microbiology of the intestinal canal can conveniently be studied in two parts: the alimentary tract without the cæca, and the cæca alone. The techniques reported in this paper were developed so that *in vivo* studies could be made on the microbiology of the intestinal flora.

It has been found practicable to remove the cæca from newly hatched chicks so that the microflora of the intestine can develop in their absence. Cæcectomy of day old chicks is described first. The study of the microflora of the cæcum is greatly facilitated by the establishment of a permanent fistula; a successful technique, which has been briefly reported [Beattie and Shrimpton, 1957], is described below.

The metabolic activity of the cæcal flora leads to the production of gases (methane, carbon dioxide, ammonia and hydrogen sulphide). The hypothesis is put forward that ecological changes within the cæcum will be reflected in changes in the metabolic activity of the microflora. A qualitative and quantitative assessment of rates of gas production has been chosen as an index of this metabolic activity and chemical techniques have been developed for this purpose.

EXPERIMENTAL

Management of Birds.—White Leghorn × Rhode Island Red cockerels and pullets have been kept in wire-floored cages and fed throughout life on a diet containing approximately 18 per cent of crude protein and 5·4 per cent of crude fibre (Table I). The temperature of the animal house was thermostatically controlled at 15° C.

TABLE I.—COMPOSITION OF DIET FED TO EXPERIMENTAL BIRDS

Feeding stuff	Composition, per cent
Ground barley	20
Ground wheat	20
Sussex ground oats . . .	10
Yellow maize meal . . .	11·75
Middlings	15
Decorticated groundnut meal .	5
White fish meal	5
Dried grass meal (lucerne, English)	5
Dried skim milk (dried whey) .	5
Dried yeast	2
Minerals + vitamin premix * . .	1·25
	100·00

* Composition of vitamin premix per lb. of diet:		
	synthetic vitamin A	2000 i.u.
	synthetic vitamin D	446 i.u.
	synthetic riboflavin	1 mg.

Anæsthesia.—Pentobarbitone (nembutal) at a concentration of 60 mg./ml., was dissolved in water containing 15 per cent (v/v) ethyl alcohol and given at 30 mg./kg. of live weight. Newly hatched chicks were injected subcutaneously; older birds were starved for 2 hr., water being available, and injected intraperitoneally. Half this dose was repeated hourly if necessary. Ether was given when deep anæsthesia was required.

Surgical Techniques

(a) *Cæcectomy.*—The anæsthetized chick was fastened, back downwards, to an operating board by its legs and wings with elastic bands. After the down on the abdomen had been plucked, the skin was washed with a solution of euflavin (1 in 10,000 w/v). The abdominal wall was incised in the middle line, the cæca lying to the right of the gizzard and below the liver. On exposure, the cæca tended to contract and expel their contents; they were then stripped from the intestine, each being cut off at the ampulla. The cæcal ducts were not ligatured before section. The abdominal wall was then closed by three cotton sutures, each passing through the skin and the muscle layers.

After operation, the chick was kept in a laboratory incubator at 37° C. until complete recovery from the anæsthetic (about 2 hr.) and then placed in a rearing brooder. No special treatment was necessary.

(b) *Preparation of Cæcal Fistula.*—Cockerels or pullets at least 2 months old have been used. The anæsthetized bird was tied, back downwards, to an operating board by its legs and wings, the body of the bird being held firm by two sand-bags placed one on each side. After the abdomen and inner sides of the legs had been plucked, the skin was washed with euflavin (1 in 10,000 w/v) and covered with a sterile lint cloth. The corners of the cloth were held in place with stitches through the bird's skin and a slit was cut through which the operation may be made. The abdominal cavity was opened to the left of the centre line so that the cæca, which are usually covered by the duodenal loop, can be drawn out through the wound with some of the small intestine. One cæcum was then mobilized and an anchor suture (of cotton) inserted into the muscular tip. The second cæcum and the intestine were replaced in the visceral cavity. A stab wound was next made through the skin of the left side of the abdomen about 5 cm. from the middle line and a tunnel was prepared, leading to the first incision, between the muscles of the abdominal wall and the skin. The mobilized cæcum was then drawn through the tunnel and its tip sutured to the skin. Both wounds were closed using cotton sutures. The abdominal wall was separately sutured, care being taken to close the wound sufficiently to prevent hernia without constricting the exteriorized cæcum.

After 10 days, when healing was complete, the tip of the exteriorized cæcum was opened. The fistula was made permanent by suturing the cæcal mucosa to the skin with radial stitches. Because the exposed cæcal contents quickly dry to form a hard cap, no cover or plug is necessary.

In this way the cæcum and abdominal cavity were never opened at the same time so that there was no possibility of cæcal contents entering the peritoneum, which, in our experience, always results in the death of the bird.

Chemical Techniques

(a) *Determination of Gaseous Metabolites.*—The gaseous products of cæcal metabolism were removed from the cæcum through a cannula in a stream of nitrogen which was passed through a specially designed micro-apparatus where alkaline gases, hydrogen sulphide, acidic gases, except hydrogen sulphide, and methane were determined simultaneously. The pH of the cæcal contents was determined *in situ*. The design of this apparatus is shown diagrammatically in fig. 1. All glass junctions are standard (B. 10 or B. 14); polythene tubing is used where flexibility is necessary. Each gas absorber consists of one gas wash bottle head, "Dreschel type", fitted into a test tube 10 cm. long and 1 cm. internal diameter. The inlet tube is drawn out so that its tip is less than 0·5 cm. from the bottom of the test tube, thus releasing small bubbles of gas. Gas absorption was not improved by packing the test tube with glass beads or rings. Nitrogen, at a controlled pressure and flow, about 20 mm. Hg and 0·5 l./hr. respectively, is passed through a polythene tube (internal diameter 1 mm.) carried within the cannula into the cæcum. After circulation through the cæcal contents the nitrogen is withdrawn through the cannula together with the gaseous products of the cæcal metabolism. Using a negative pressure, about 30 mm. Hg, the gases are drawn through the series of absorbers where their concentration is determined by consecutive titrations. The contents of each absorber and the gases they are designed to estimate are shown in Table II. The time taken for the indicator in each absorber to change is recorded so that the rate of production of each gas is determined directly. When the contents of an absorber are neutralized they are replaced immediately.

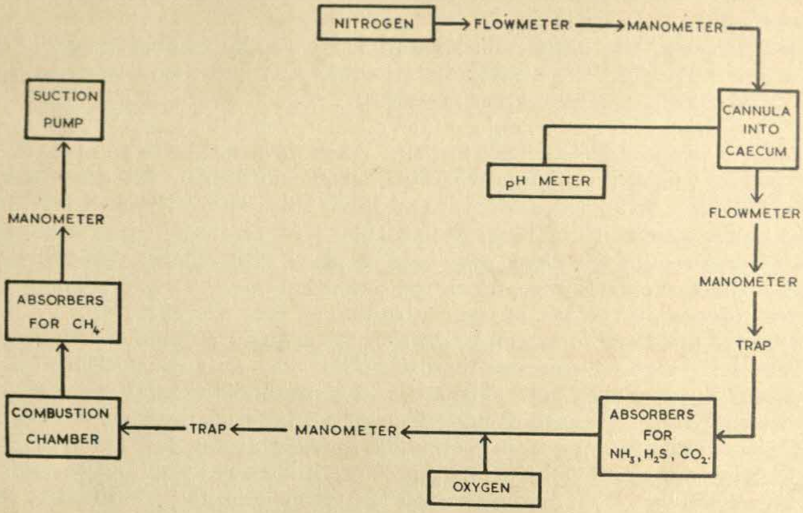

Fig. 1.—Diagrammatic representation of apparatus for studies on the metabolism of the cæcal microflora of the fowl *in vivo*.

Because the gas stream is passed through a chain of absorbers arranged in series, an amount of each gas, proportional to its solubility coefficient, will be retained by each absorber through which the gas is passed. However, the amounts so retained are negligible as the concentrations of the gaseous metabolites in the carrying stream of nitrogen are small (< 0·03 per cent) and a steady state is quickly established.

Table II.—Contents of Absorbers (Volume in each Absorber = 2 ml.)

Absorber No.	Absorbant	Concentration	Indicator	Gas detected
1	HCl	N/1000	Methyl orange	Alkaline gases, calculated as NH_3.
2	I_2 (in alcohol)	μ molar/5	None (iodine decolorized)	H_2S.
3	NaOH	N/400	Phenolphthalein	Acidic gases, calculated as CO_2.
4	$Zn(CH_3COO)_2$ (trap)	2 per cent	None	Trap for H_2S.
5	NaOH (trap)	N/10	Phenolphthalein	Trap for acidic gases.
6	NaOH	N/400	Phenolphthalein	CO_2 from combustion chamber, calculated as CH_4.
7	NaOH	N/10	Phenolphthalein	Trap for CO_2 from combustion chamber, calculated as CH_4.

(b) *Determination of pH.*—The circuit used for the simultaneous determination of pH is shown in fig. 2. A small glass "Miniature Probe Electrode",* maximum diameter 4 mm. is housed in a rotating holder so that it may be introduced either into a washing compartment or into the cannula and thence the cæcum without opening the gas circuit. Connection with the calomel electrode is established with a bridge of saturated potassium chloride stabilized with 0·5 per cent agar within a polythene tube, internal diameter 1 mm. [Dobson, 1956]. To avoid difficulties of temperature correction, readings are made of the potential of the system, the corresponding pH being calculated from a calibration curve.

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* W. G. Pye & Co., Cambridge.

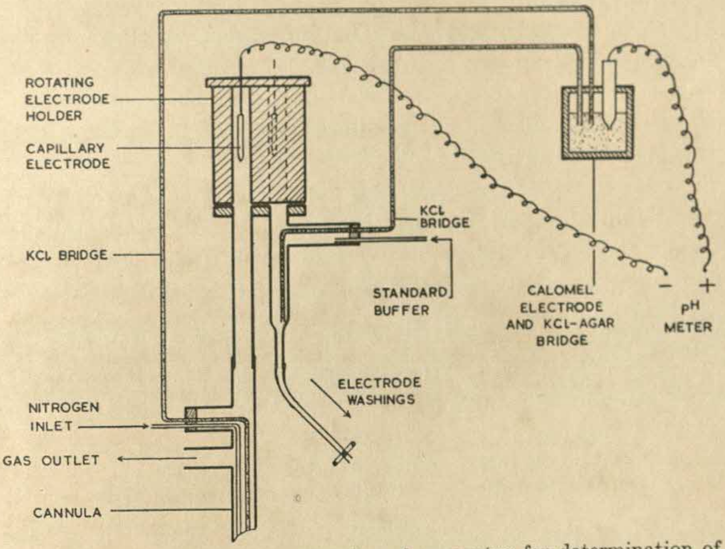

FIG. 2.—Diagrammatic representation of apparatus for determination of the pH of the cæcal contents of the fowl *in vivo*.

Determination of Temperature.—A thermocouple of 32 S.W.G. copper/constantan was used. The tip of the thermocouple was sheathed with cellophane to reduce the risk of piercing the cæcal wall and it was calibrated under the conditions of the experiments.

RESULTS

Post-operative Condition of Birds

Cæcectomized Birds.—After 12 weeks, twenty cæcectomized cockerels were slightly heavier than twenty control birds, but the difference was not significant. When the birds had been killed, all the cæcectomized and six of the control birds were dissected and the lengths of their intestines were measured (Table III).

TABLE III.—LENGTHS IN CENTIMETRES OF INTESTINES OF CÆCECTOMIZED AND CONTROL BIRDS AT 12 WEEKS OF AGE

	Cæcectomized (19 birds) * range	Control (6 birds) range
Small intestine + large intestine . .	122–191	135–151
Large intestine . .	7·5–11	6·4–10
Cæcum . . .	0·3–2·2	14–19

* In one bird the cæcum was not successfully removed.

In the cæcectomized birds, the rudimentary cæca were less than 2·2 cm. long, some being only 0·3 cm. long, compared with 14–19 cm. for the cæca of control birds. The rudimentary cæca (fig. 3) were not differentiated into duct and sac and their lumens were empty. The operation was therefore a success as no functional cæcum was present at any stage of development after hatching. Since, the cæcal flora is undeveloped at hatching [Lev and Briggs, 1956 *a*, *b*] and not typical of the adult bird until feeding begins, the flora of the large intestines of the cæcectomized chicks must have developed in the absence of cæcal organisms. The microflora of the large intestine may therefore be studied from fæcal collections.

In earlier experiments, the cæca had been removed after the ampullæ had been ligatured and the majority of these birds had functional cæca at one month of age. This difficulty was overcome when the cæca were sectioned close to the intestine, as described here, which is not possible when the ampullæ are ligatured.

TABLE IV.—RECOVERY OF CARBON DIOXIDE FROM GAS MIXTURES BY MICRO-APPARATUS

Per cent of carbon dioxide in gas mixture	Rate of flow of gas mixture ml./hr.	Per cent recovery of carbon dioxide
5	350	11
0·5	420	9
0·03	950	37
0·03	400	78

Fistulated Birds.—Cæcal fistulæ have been prepared in nine birds with no deaths. Two were pullets and egg production for a 3-month period was normal.

Gaseous Metabolites detected in the Cæca of Fistulated Birds.—The gases detected in the greatest amounts were methane and acidic gases, calculated as carbon dioxide, the total order of production being 100 μl./hr. at N.T.P. per cæcum. Small amounts of hydrogen sulphide and alkaline gases, calculated as ammonia were also detected.

When the efficiency of the alkaline absorbers was tested at several rates of flow with nitrogen-carbon dioxide gas mixtures (Table IV) a satisfactory recovery, of the order of 80 per cent was achieved only when the total amount of carbon dioxide passed in 1 hr. did not exceed 120 μl. This condition was satisfied in the present experiments.

The titrimetric determination of hydrogen sulphide with alcoholic iodine was compared with a colorimetric method in which the hydrogen sulphide was measured after absorption in 2 per cent zinc acetate solution, by reaction with dimethyl-p-phenylene diamine (Lauth's reaction) following the method of Almy [1925] using appropriately reduced quantities of reagents. The results obtained by both methods from the cæcum of an anæsthetized bird are compared in Table V. The more convenient titrimetric method was used in subsequent experiments.

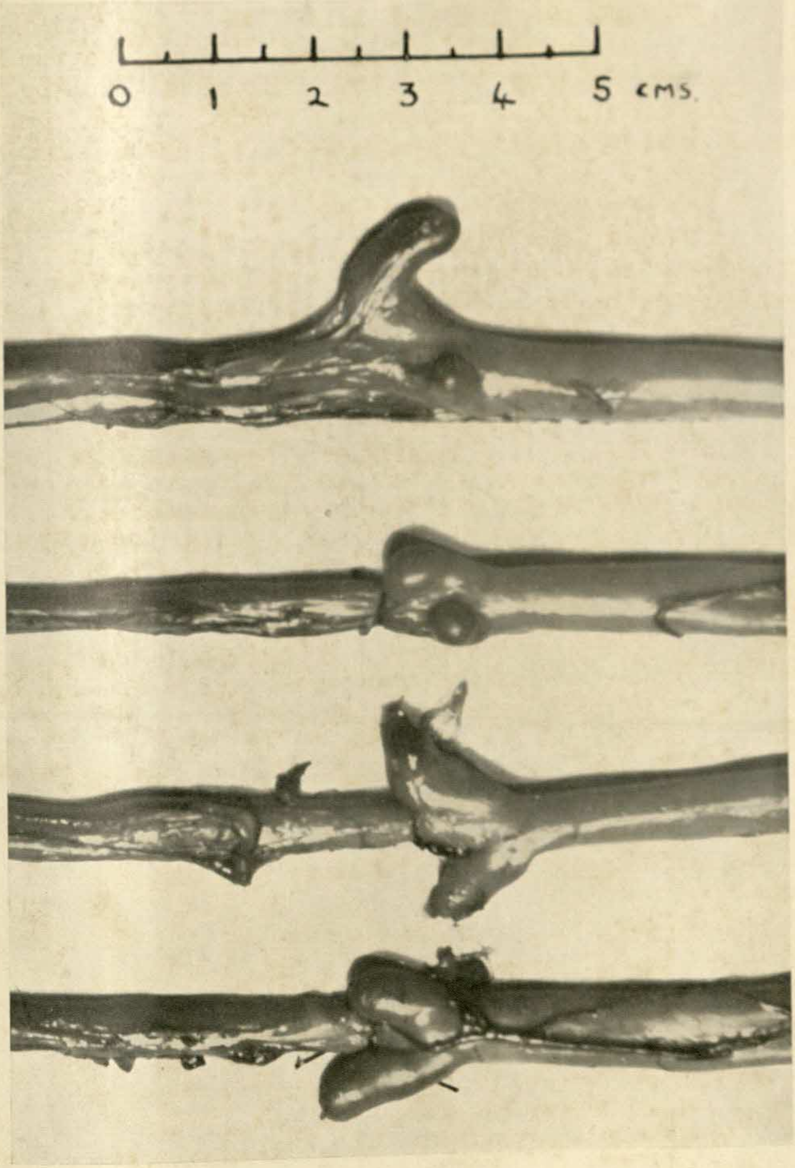

FIG. 3.—"Rudimentary" caeca from 12 week old cockerels which had been caecectomized at 1 day old.

Observations on a cæcum of one bird over a period of 5 hr. are shown in fig. 4. Both methane and carbon dioxide were produced uniformly and a steady state was established in the first 2 hr. of the experiment. The mean rates of gaseous production were then 85 µl./hr. for methane and 47 µl./hr. for carbon dioxide. Small quantities of hydrogen sulphide and ammonia were also detected, but they are not shown in fig. 4.

Table V.—Comparison of Consecutive Colorimetric and Titrimetric Determinations of Rate of Production of Hydrogen Sulphide

Time (P.M.)	Analytical method	Period min.	Total amount of sulphur µg.	Rate of collection of sulphur µg./min.
12.57–2.37	Colorimetric	100	36	0·36
2.37–2.55	Titrimetric	18	5·3	0·29
2.55–3.15	Colorimetric	20	6·7	0·33
3.15–3.29	Titrimetric	14	5·3	0·38
3.29–3.49	Colorimetric	20	7·5	0·38

Whilst the amounts of gas produced by the same cæcum of one bird have remained approximately constant, we found that there were considerable differences between birds, fed on the same diet and of like ages; the relative proportions of the cæcal gases to one another were, however, similar. Methane and acidic gases calculated as carbon dioxide predominated, with small amounts of hydrogen sulphide and alkaline gases calculated as ammonia.

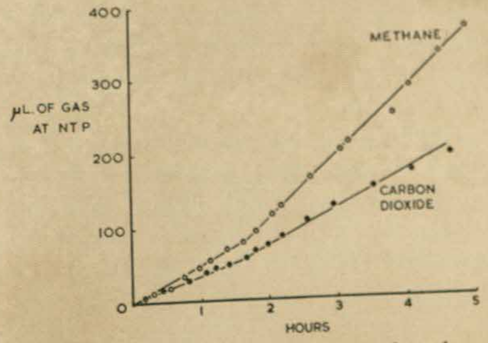

Fig. 4.—Production of methane and carbon dioxide from one cæcum of an anæsthetized cockerel.

pH of Cæcal Contents.—The pH of cæcal contents determined *in vivo* ranged between 8·5 and 6·7. Determinations made *in situ* 18 hr. or more after death were lower, being between 5·9 and 6·2.

Whenever the micro-electrode was inserted into a previously undisturbed cæcum *in vivo*, a pH gradient was observed, the highest pH being at the tip and the lowest close to the cæcal duct. For example, in the cæcum of one adult cockerel the pH at the tip was 7·6 and 8 cm. from the tip was 6·7. In another cockerel the change over the same distance was from 8·5 to 7·9. It was not possible to repeat the observations during the same experiment, presumably because of the mixing of the contents necessarily resulting from the insertion and withdrawal of the micro-electrode. When a micro-electrode was inserted repeatedly during the same experiment no gradient of pH was observed and the value of the pH was slightly lower than that first recorded at the tip. No gradient of pH was observed in the cæca of birds which had been dead for 18 hr. or more.

Temperature of Cæcal Contents.—A small increasing temperature gradient was observed between the opened tip of a cæcum of a live anæsthetized bird and the cæcal duct. In a typical experiment temperature of 35·9° C., 39·0° C. and 39·5° C. were recorded at depths of 2·2 cm., 6·2 cm., and 10·4 cm., from the opened tip.

DISCUSSION

In spite of the microbiological activity within the cæca of normal birds, cæcectomized birds have grown at least as well as the controls and, at 12 weeks of age, tended to be heavier. This is in agreement with the observations of Sunde, Cravens, Elvehjem and Halpin [1950]. A satisfactory rate of growth in the absence of cæca does not appear to be the result of any increased microbiological activity in any other part of the intestine, since the dimensions of the large intestine and of the entire alimentary canal were similar to those of the control birds. Nevertheless it cannot be concluded from these experiments that the metabolism of the cæcal microflora has no effect upon the birds, but only that under standardized conditions it had no net effect. Possibly some changes in the metabolism of the cæcal microflora may result in a net advantage to the bird.

The major microbiological activity of the cæcum is fermentative rather than putrefactive since the principal gaseous products of metabolism are methane and carbon dioxide, hydrogen sulphide and ammonia being minor constituents. The composition of cæcal gas is thus similar to that of sheep rumen gas [Quinn, van der Wath and Myburgh, 1938] where small quantities of nitric oxide were also found. The type of cæcal fermentation, however, is probably a result of the composition of the diet. For example, Shrimpton [1958] has found that when maize is the major constituent of the diet, little methane is formed.

The pH of the cæcal contents determined *in situ* has not been less than 6·7, but previously reported values, all from expressed contents, range from 5·4 [Buckner, Insko and Henry, 1944] to 8·20 [Olson and Mann, 1935], Farner [1942] quoting a mean value of 5·60–5·83. Although the diets of these birds are not reported in all cases, it is suggested that part of this variability in cæcal pH is an artefact resulting from the removal of the cæcal contents from their environment within the living cæcum.

Because the *in vivo* pH of the cæcal contents changes, from the tip, where it is highest, through the length of the cæcum to the cæcal duct, where it is lowest, it is unlikely that these contents are homogenous. Also, an empty cæcum has never been observed by us, even when an operation has immediately followed the expulsion of cæcal contents. It is therefore improbable that all the contents are removed at every cæcal evacuation and at least possible that the cæcum contains at any one time, regions where the bacterial fermentation is proceeding from a relatively long established microflora and others, possibly near to the cæcal duct, where the fermentation results from a microflora only freshly introduced from the small or large intestine. Thus, from the viewpoint of microbial ecology, the cæcum may contain several distinct micro-populations.

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SOME EXPERIMENTS IN FLUORESCENT MICROSCOPY DESIGNED TO ELUCIDATE THE FATE OF THE LYMPHOCYTE.

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The fate of the lymphocyte has been studied by means of transfused thymocytes labelled with acridine orange. The following points have been established: (1) In some cases, there is a limited recirculation of transfused cells. (2) The majority of injected cells are removed from the blood and destroyed within 2 hr. (3) The transfusion of living thymocytes or thoracic duct lymphocytes is followed by an increase in the thoracic duct cell output.

INTRODUCTION

THE fate of the enormous number of lymphocytes which flow continuously into the blood from the thoracic and right lymph ducts has been the subject of much research and discussion [Yoffey and Courtice, 1956]. In the rat, if the thoracic duct alone is considered, the number of cells entering the blood daily is about 7×10^8, whilst the total number of lymphocytes circulating in the peripheral blood is fairly constant at 7×10^7 (see under results). Yoffey [1936] has called the ratio of cells entering the circulation to the number already there, the Daily Replacement Factor [D.R.F.]. This varies from 0·5 to 3·5 in the cat [Sanders, Florey and Barnes, 1940], and from 2 to 24 in the rat [Reinhardt, 1946; Mann and Higgins, 1950; Whalen and Widdicombe, 1956]. Our figures (Table I) of about 10 for the rat agree well with those obtained by Gowans [1957]; as there are sources of blood lymphocytes other than the thoracic duct, the true D.R.F. must be somewhat higher than this. The number of cells involved is very large and we were attracted by the possibility of following them to their ultimate destruction by the use of fluorescent tagged lymphocytes, as suggested by Farr [1951].

There have been many suggestions as to the ultimate fate of the lymphocytes entering the peripheral blood [Fichtelius, 1953; Yoffey and Courtice, 1956].

1. They may die and be broken down in the blood. On the whole the evidence is against this [Wiseman, 1932; Bunting and Huston, 1921; Drinker and Yoffey, 1941].

2. They may re-enter the lymph, *i.e.* take part in a closed circuit circulation. Yoffey and Drinker [1939], believed that recirculation can only form a small part of the thoracic duct flow, since there is a thirty fold difference in the cell content of peripheral and intermediate lymph. However, as pointed out by Fichtelius [1953], it is possible for lymphocytes to leave the blood in the nodes and so enter the intermediate lymph without ever having been in the peripheral lymph.

3. They may enter the hæmopoietic tissues and transform into other types of cells. This could account numerically for all or most of the thoracic duct output, provided there is no appreciable cell multiplication in the bone marrow. It would mean that the lymphocyte is a highly potent and so presumably undifferentiated cell. Farr [1951] concluded that at least some of the lymphocytes leaving the circulating blood go to the bone marrow where they transform into cells of the granulocytic series.

TABLE I.—SHOWING WEIGHTS AND INITIAL THORACIC DUCT COUNTS OF FORTY-SEVEN RATS

Expt. No.	Weight (g.)	Thoracic duct count/cu. mm.	Expt. No.	Weight (g.)	Thoracic duct count/cu. mm.
7	490	40,000	98	325	35,200
8	420	14,000	99	440	48,400
14	320	30,000	100	320	16,700
15	210	41,600	101	200	33,000
16	280	68,000	102	250	25,300
17	100	18,900	103	340	24,700
24	100	66,000	104	500	33,600
25	100	33,600	105	470	54,600
26	220	68,000	106	280	40,200
32	190	41,600	107	330	40,800
39	450	79,000	108	280	37,800
47	250	34,000	109	270	55,800
52	285	13,700	110	280	41,400
56	120	26,000	111	260	14,300
83	400	29,000	112	250	64,000
84	500	60,400	113	240	43,600
85	80	27,400	114	305	57,200
87	200	32,000	115	210	48,200
88	240	54,000	116	266	68,600
89	230	47,000	117	200	52,600
94	204	81,200	118	150	63,600
95	210	56,800	119	200	39,000
96	170	44,600	120	180	42,600
97	370	17,100	Av. weight	270	42,700

(Average flow 0·7 ml./hr. range 0·5–1·0 ml./hr.)

4. They may be excreted into the intestinal lumen [Bunting and Huston, 1921].

5. They may degenerate or be broken down in specific organs or tissues. The present work shows that in some individuals there is at least a limited recirculation of transfused cells, but that the greater number are sequestrated and degenerate in the lung, kidney, liver and spleen. The continuous addition of living lymphocytes to the blood appears to be essential to the maintenance of the thoracic duct output of cells. The conclusions drawn by others will be discussed in terms of our own results.

MATERIALS AND METHODS

Approximately 125 experiments were done, using 200 Wistar albino rats (150–250 g.). They are hardy and stand up well to operations. Animals were anæsthetized with intraperitoneal nembutal (60 mg./kg.) repeated as necessary.

Positive pressure respiration, when required, was controlled by an escape valve and a water manometer.

The thoracic duct was exposed in the region of its junction with the cervical duct and distended by passing a fine ligature round it. An incision in the duct wall then permitted the approximation of a glass cannula for the collection of the lymph. Even in small animals this technique could be completed within 20 min. The initial flow of lymph was discarded and the flows were measured at half hourly intervals for at least 2 hr. Counts were made on pooled 30 min. samples.

Cells from the thymus have been injected into the femoral vein, aorta, and occasionally into the left atrium or left ventricle. The injections were made slowly at a rate of approximately 0·1 ml./min. Cells from the mesenteric lymph nodes, spleen and thoracic duct have also been transfused. Thymus glands after removal from exsanguinated animals were placed immediately in pyrogen-free Tyrode solution adjusted to pH 7·5 by CO_2. A single cell suspension was obtained by mincing the gland in a small quantity of Tyrode solution with a mechanically rotated knife consisting of a flat blade rotated in a stainless steel cylinder.* After mincing for 1 min. at 2000 r.p.m. the volume was made up to 10 ml. with further Tyrode solution. The suspension was allowed to stand for 10 min. to allow clumps and large debris to settle. The supernatant was next pipetted off and acridine orange in buffered saline pH 7·0 was added to a final concentration of 1: 50,000. The choice of this dye will be discussed later. The stained suspension was spun down and the supernatant. pipetted off. The final suspension was made up to ½–1 ml. with Tyrode solution or pyrogen-free saline and had a cell density from 100,000 per cu. mm. to 500,000 per cu. mm. Colorimetric estimation showed less than 1 part in 10^7 free dye.

Before injection the cells were examined on a fluorescent microscope for depth of staining and to ensure the absence of clumping, using a N.A. 0·95 oil immersion objective and a sphere cardioid dark ground condenser. The source was a 1/4 k.w. high pressure Mercury Arc used in conjunction with a Chance's Ox 7 glass filter. Residual visible light was removed with a liquid filter consisting of a 3 per cent aqueous solution of copper nitrate.

In the irradiation experiments a Cobalt therapy unit was used as a source γ radiation. Rats in perspex cages were irradiated for approximately 1 hr. to give a total body dose of 800–1000r.

RESULTS

Thoracic Duct Recirculation.—In fourteen experiments, suspensions of acridine orange stained thymocytes were injected intravenously into rats in which the thoracic duct had been cannulated. The thoracic duct lymph was examined half hourly for fluorescent cells. In five of the experiments brightly fluorescent cells were found. The proportion of stained cells was variable but was never greater than 20 per cent of the total in any sample. It is difficult to obtain a more precise figure than this as unstained cells are not visible under the fluorescent microscope. Furthermore, the stained cells only appeared between 1½ and 2 hr. after injection: and this was independent of the number of cells injected ($50–500 \times 10^6$). There was no apparent correlation between the presence of stained cells and the degree of consanguinity between donor and recipient. After intravenous transfusion of stained thymocytes, the thoracic duct cells increased by an average of 8000 cells/cu. mm. (Table II). This increase in output was not sustained; it was irrespective of the subsequent appearance of stained cells in the lymph or whether the suspending fluid was saline or Tyrode solution. A typical response is shown in fig. 1. To demonstrate this point better, thoracic duct cells were counted on five anæsthetized normal animals for 4 hr. after operation. The count fell to half the original value. Injection of stained or unstained thymocytes at this time rapidly restored the count, yet no fluorescent cells were seen. A typical response is shown in fig. 2.

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* We wish to thank the Director, M.R.C. Radiobiological Unit, Harwell, who sent us a copy of the "mincer" used in that Unit for making single cell suspensions.

Table II.—Ten Experiments carried out to Show the Increase in the Thoracic Duct Cell Count following the Intravenous Injection of Thymocytes

Expt. No.	Thoracic duct count before inj. per cu. mm.	Thoracic duct count max. after inj. per cu. mm.	Increase in thoracic duct count per cu. mm.	Response time min.
11	11,600	15,900	4,300	90
12	19,000	26,000	7,000	60
25	24,400	32,000	7,600	90
52	13,700	23,400	9,700	60
87	32,000	55,000	23,000	60
94	72,000	90,200	18,200	20
95	52,000	57,500	5,500	30
96	37,000	41,000	4,000	15
97	14,600	19,400	4,800	15
98	48,000	58,000	10,000	60

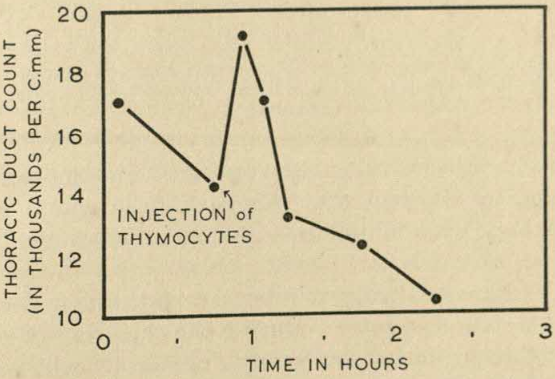

Fig. 1.—The response of the thoracic duct cell count to the intravenous injection of living thymocytes soon after cannulation.

The fluorescent cells appearing in the thoracic duct might be: (1) the injected cells, (2) cells of the recipient stained by the residual dye in the suspending fluid, or (3) cells stained by the release of dye from injected cells.

As the amount of free acridine orange injected did not exceed 0·5 ml. of 0·00001 per cent acridine orange, 0·5 ml. of ·001 per cent acridine orange, *i.e.* 100 × the above concentration, was injected intravenously following thoracic duct cannulation. No fluorescent cells appeared in the lymph. When the concentration was increased to 0·01 per cent faintly fluorescent cells appeared in the thoracic duct lymph within half an hour. A further one hundred fold increase did not affect the depth of staining of these cells. Thus the amount of dye, free and fixed, injected could not account for the brightly fluorescent cells seen after transfusion.

In the positive experiments, the number of fluorescent cells which appeared in the thoracic duct lymph was estimated to be of the order of 10 million out of the 200 millions injected. Thus thoracic duct recirculation can only account for about 5 per cent of the injected cells. Examination of the peripheral blood 1 hr. after the injection accounted for only a few more of the injected cells, as only an occasional fluorescent lymphocyte could be found. Although enough cells had been injected to treble the peripheral blood lymphocyte count, no increase was detected. Aortic blood sampled during and after the injection likewise contained few fluorescent cells. This could be explained by the fact that they had been caught in the lungs in which large numbers were found in animals killed immediately after the transfusion.

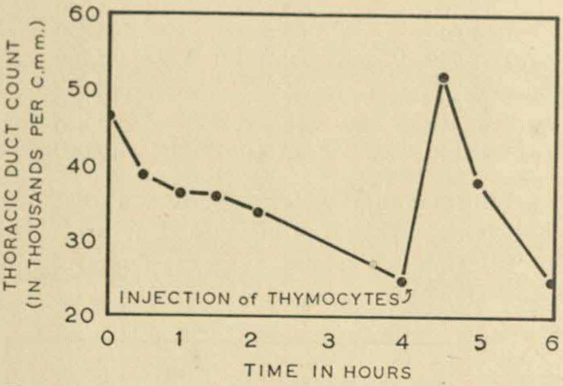

FIG. 2.—The response of the thoracic duct cell count to the intravenous injection of living thymocytes 4 hr. after cannulation.

Trapping by the Lungs.—Under nembutal anæsthesia with positive pressure respiration, the chest was opened. During the injection of stained cells, the lungs became brightly coloured and many fluorescent cells were seen in both squashes and fresh frozen sections. Subsequent biopsies showed increasing numbers of fluorescent cells with red nuclei, a colour change associated with death of the cell. In addition, red fluorescent granules appeared in many of the parenchymatous cells. Within 2 hr. of the injection no transfused cells could be detected in the lung.

To determine whether it was the few cells that managed to pass through the capillaries of the lung which appeared in the thoracic duct, similar injections of stained cells were made into the aorta and in no case did stained cells appear in the thoracic duct. It would appear, therefore, that trapping by the lung was an essential part of the pathway that these fluorescent injected cells took to the thoracic duct. The lungs have been said to drain chiefly into the right lymph duct, accordingly it was cannulated and dyed cells injected intravenously. No fluorescent cells were found in the lymph, thus excluding any large scale recirculation by this route. (Old and young litter mates and non-litter mates were used as donor/recipient.)

Sequestration of the transfused cells by the lungs seemed to be independent of their relationship. Similar results were obtained with autogenous, homogenous and heterogenous transfusions of both guinea-pig and rat cells. The fate of the injected lymphocytes is not a property of their site of origin. Experiments with cells from the spleen, mesenteric lymph node and thoracic duct lymph yielded similar results. The thymus was used as the source of lymphocytes for most experiments because of the difficulty of getting large numbers of fresh cells from the thoracic duct.

To exclude an antibody reaction as the cause of the sequestration and death of the transfused cells by the lungs, a number of rats were given 800r. whole body radiation and operated on 2 hr., 1 day, 3 days and 5 days after exposure. The thoracic duct was cannulated and stained thymocytes injected as before. In the one, three and five day experiments only faintly stained cells were found in the thoracic duct. As before, after 2 hr., no stained cells could be found in the recipient animal either in lung, liver, kidney, spleen, bone marrow, thymus, mesenteric lymph node or blood.

One of the principal effects of large doses of radiation is to stop mitosis. As within 2 hr. of irradiation the thoracic duct count falls from a normal value of 43,000 cells per cu. mm. to approximately 6000 cells per cu. mm. it would seem probable that output is dependent on newly formed cells. If thoracic duct lymphocytes were derived to any extent from peripheral lymphocytes, the fall in the peripheral count should precede the fall in the thoracic duct count. The reverse is the case. By the fifth day the thoracic duct count was still approximately 2000 cells per cu. mm. whilst the peripheral blood lymphocyte count had fallen to one third of that figure. After the transfusion of stained lymphocytes into irradiated rats, the thoracic duct lymphocyte count doubled within 2 hr., although none of the brightly fluorescent injected cells appeared in the lymph—a similar response to that observed with many of the non-irradiated animals.

To enable the fate of those thymocytes which occasionally reach the liver, kidney and spleen to be followed more easily, the number of cells reaching these organs was increased in the following manner. Rats were anæsthetized, the aorta tied above the bifurcation and the intestines removed. Injections of 2×10^8 cells were then made into the aorta above the ligature. During the injection, the kidney rapidly became yellow but regained its normal colour in a few minutes. Less marked changes were seen in the liver and spleen. Biopsies of these tissues were taken frequently during the next 2 hr. The disappearance of the brightly fluorescent cells was most rapid in the kidney and liver. In all cases the fluorescent cells apparently lost nuclear structure, showed red nuclear fluorescence and then disappeared as distinct entities. In the kidney the parenchymatous cells rapidly took up the fluorescent material and themselves showed definite but much fainter fluorescence. This was also observed occurring later in the spleen, but not in the liver.

Similar results were obtained after injection of the cells into the left ventricle without the removal of the intestines. In all experiments some stained cells could be seen in the lungs.

Thus the majority of the injected cells reaching the lung, liver, kidney and spleen were sequestrated and soon died. To determine whether this was a property of the cells or of the body tissues, dyed cells were kept in Tyrode solution and in blood for up to 5 hr. at both room (18° C.) and body (37° C.) temperature without showing signs of degeneration. Thymocytes were examined on a warm stage at 37° C., by phase contrast microscopy. When suspended in Tyrode solution they became spherical and showed only minor pseupodial activity. However, when they were incorporated in a plasma clot almost all showed the short bursts of typical lymphocyte mobility described by Ebert, Sanders and Florey [1940]. Stained and unstained cells behaved similarly. Thymocytes prepared for injection were tested for viability [Shrek, 1943] and in both stained and unstained suspensions more than 80 per cent of the cells were alive 2 hr. after preparation. Subcutaneous depots of stained cells remained apparently normal for at least 5 hr. after injection—the limit of our observations, and in none of these experiments was any fading of the fluorescence seen. Furthermore, injection of sufficient dye intravenously into rats to give a total body concentration of 1 : 20,000 had no apparently deleterious effect.

DISCUSSION

The interpretation of the results depends on the nature of the injected cells. Mostly they were thymocytes that had been separated by mincing and then stained in a solution of acridine orange. Clearly this raises two issues:

1. *Identity of Thymocytes and Lymphocytes.*—Injected thymocytes behaved in the same way as cells from the spleen, mesenteric lymph nodes and thoracic duct lymph. There were no differences in the morphology of the stained cells when examined by fluorescent microscopy. Ultra-violet and phase contrast microscopy have failed to differentiate between lymphocytes from the various sites. There is substantial evidence in the literature to corroborate these findings [Kindred, 1942; Andreasen and Ottesen, 1945; Andreasen and Christensen, 1949; Osogoe and Hitachi, 1950; Drinker and Yoffey, 1941 and Fichtelius, 1953].
2. *Effect of Labelling with Acridines.*—Subcutaneous depots of stained cells remained normal for many hours after injection and incubation at 37° C., and the Schrek test confirmed their vitality. The use of the diamino-acridines as supravital dyes has been investigated by de Bruyn *et al.* [1950]. Acridine orange provided a very adequate stain of low toxicity. It also exhibited differential fluorescence and was readily available in purified form [Armstrong, 1956; Bishop, 1957]. Lymphocytes stained with concentrations of the dye not greater than 1 : 20,000 clearly showed this phenomenon. The nucleus

> of the cell fluoresced a bright yellow-green whilst the cytoplasm appeared as a red granular structure. At greater concentrations of acridine orange a proportion of cells became orange-red whilst in stronger solutions the nucleus became brightly red-fluorescent. Evidence that the nuclear fluorescence of cells changes with their death has been published by Strugger [1949]. We suggest that lymphocytes behave in a similar manner. Irradiation with ultraviolet light or prolonged storage increased the proportion of red nucleated cells in stained lymphocyte suspensions. Using this criterion the injections showed only an occasional degenerate cell.

Farr [1951] used fluorescent labelled cells and concluded that at least one of the fates of transfused lymphocytes was their conversion into myelocytes. Even at the low concentrations of 3–6 diamino-10-methyl-acridinium hydrochloride which Farr used, the dye produced an intense non-differentiating fluorescence. Diaminoacridines have a strong affinity for nucleoprotein, and our observations suggest that following the apparent degeneration of transfused cells the parenchymatous cells themselves appear palely stained. It is possible that the myelocytes that Farr observed were in fact cells which had taken up stain—released or still fixed to the nucleoprotein. As mitoses can be observed in marrow, only a small percentage of the daily output of thoracic duct lymphocytes would in any case be required for such transformations.

Increased thoracic duct cell output following the transfusion of lymphocytes occurs only with living cells [Gowans, 1957]. The fact that this response invariably followed transfusions of acridine orange stained cells was additional evidence of their viability.

In one third of our experiments there was a limited recirculation of lymphocytes via the thoracic duct, although it did not amount to more than 5 per cent of the total thoracic duct output. The limited number of experiments in which the right lymph duct was cannulated showed that there was a no greater recirculation by that route. In the irradiation experiments, the thoracic duct output fell rapidly before the peripheral blood count was appreciably affected. The rapidity of this fall indicated that the cells in the thoracic duct lymph were newly formed. The path of such recirculation as there is must be via the lung, as we were unable to detect it following intra-aortic injections. The period of recirculation was remarkably consistent at 1½–2 hr., which was presumably the time taken for the active migration of the lymphocytes from the lung. Although our maximum figure of 5 per cent recirculation agrees well with that of Yoffey and Drinker [1939], and Andreasen and Ottesen [1945], it would seem, because of the large number of cells injected, to be of the nature of an overflow rather than a normal physiological pathway. Although the recirculating cells showed no characteristic features, biological differences may have existed in them as suggested for blood lymphocytes by Wiseman [1932], Ottesen [1953] and Thorell [1945].

A considerable proportion of the transfused cells were held up in the lungs. The sequence of colour changes in these cells, and their disappearance after

2 hr. suggested their early senescence and death. The radio isotope technique of labelling does not enable one to differentiate between cell debris and intact cells, nevertheless Weisberger's [1951] estimate that 28 per cent of the radioactivity contained in the labelled cells remains in the lungs, 23 per cent in the liver, 4 per cent in the spleen and 5 per cent in the kidney, fits in well with our experience. Bierman *et al.* [1955] using simultaneous arterial and venous catheterization found that "the pulmonary circulation is capable of withdrawing vast numbers of cells" and states that "these large numbers of cells are not returned to the blood in a similar fashion". He suggests that sequestration and excretion by the lungs, liver, kidney and gastro-intestinal tract is a normal mechanism which "plays a purposeful rôle as an integral part of the physiology of the leucocyte in normal man". Farr [1951] found transfused cells in the blood for up to 90 min. and in the lymphoid tissue and bone marrow but not the lung after 2 hr. We were unable to find fluorescent cells in the blood for longer than 1½ hr., but could always find them in the lung during the first 1–2 hr. after injection. Unfortunately, Farr did not carry out any biopsies in the first 2 hr. of his experiments. The only fluorescence that we could find 2 hr. after the injection was the faint background fluorescence of the parenchyma of the kidney and spleen that occurred when the original injected cells were broken down. Farr found that cells from a different species (guinea-pig to rabbit) were treated differently, as he was never able to find any heterogenous cells subsequent to transfusion. Our rats reacted to injected guinea-pigs' lymphocytes in exactly the same way as autogenous injected cells.

Whilst we were able to find the occasional brightly stained cell in lymphoid tissue and bone marrow after transfusion, it was never possible to find live donor cells in the recipient 2 hr. after either intravenous or intra-arterial injection. However, faintly stained host cells were present at this time, particularly in the spleen. The reason transfused cells were held up and destroyed was not due to consanguinity between the donor and recipient animals as autogenous and heterogenous cells were treated alike; nor was it due to the source of the cells, spleen, thymus, thoracic duct, etc. Antibody reactions were unlikely within such a short time of the injection in unimmunized animals and the results of autogenous transfusions and the radiation experiments excluded such reactions.

It has been suggested that lymphocytes have a blood life of days rather than hours [Osgood, 1952, 30 days; Ottesen, 1954, 3 days and 100–200 days; and Hamilton, 1957, by deduction 21 days]. With the exception of Ottesen's two experiments all these determinations have been made on cases of lymphatic leukæmia using radioactive isotope labelling. Such life spans cannot be reconciled with Daily Replacement Factors greater than unity without postulating extravascular recirculation of the cells or re-utilization of their labelled D.N.A., *i.e.* recirculation of the whole or part of the lymphocytic nucleic acids. As Hamilton [1957] points out "the present biochemical evidence does not permit a decision whether the prolonged retention of isotopes (in the blood) reflects the survival of some cells for a long time or such a re-utilization". Whilst a maximum life of 2 hr. for rat lymphocytes in the circulation fits in well with a Daily Replacement Factor of ten or more, identical cells remained alive in subcutaneous tissue depots or tissue culture for much longer periods.

The rapid rise in the thoracic duct output of cells following the intravenous injection of stained or unstained cells occurred within 1 hr. and was not the recirculation of the injected cells. Gowans [1957] found that to maintain the thoracic duct cell output with a thoracic duct fistula, it was necessary to re-infuse live lymphocytes continually. Dead cells, however killed, had no effect on the thoracic duct output. To explain his results he put forward three possibilities: (1) the cells may die in the lymph nodes and release in them substances necessary for new cell production, (2) the reinfused cells may recirculate, (3) a small number of the reinfused cells may multiply and repopulate the lymphoid tissue. He was unable to choose between these three, but, from our experiments, the first is the most likely. The rapidity of the response to injected cells (fig. 2) excluded (3) as a possibility, as mitosis would not be fast enough to produce the response in the time available. As the injected cells were firmly tagged the second possibility could also be excluded. Hence we suggest that it is the death of transfused cells in the tissues of the host (not necessarily in the lymph nodes) that, directly or indirectly, is the stimulus for lymphocyte production or release.

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THE EFFECT OF OLIVE OIL AND OLEIC ACID ON GASTRIC SECRETION IN THE RAT.

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In the rat the inhibitory effect of olive oil on spontaneous gastric secretion is weak and erratic. Large amounts of oil have to be used. Previous hydrolysis of the oil markedly enhances the inhibitory effect. Oleic acid and sodium oleate are potent inhibitors of both spontaneous and insulin-stimulated gastric secretion.

Draining the upper intestinal juices reduces, but does not abolish, the inhibitory effect of sodium oleate on insulin-stimulated gastric secretion. Distension of the duodenum with liquid paraffin reduces spontaneous as well as insulin-stimulated gastric secretion. Distension of the jejunum or ileum does not have this effect. Distension of the duodenum with sodium oleate as well as with water reduced gastric secretion. There is a positive correlation between the inhibitory effect and the distending pressure. At the same pressure sodium oleate is more potent in depressing gastric secretion than is water.

The inhibitory effect of fat on gastric secretion has been known since the classical experiments of Beaumont [1838] and Ewald and Boas [1886]. Further experimental work by Szokolov [1904] and Lönnquist [1906] showed that the effect was due to contact of fat with the mucosa of the small intestine. Products of lipolysis such as fatty acids and soaps were supposed to have a stimulating effect on gastric secretion. This view was based on the experiments of Piontkowsky [1904] and Babkin [1905], who showed that the introduction of sodium oleate through the duodenal fistula of a dog caused secretion from "the little stomach", and on the experiments of Ivy and McIlvain [1923–4], who obtained secretion from dogs with fundic pouches after the introduction of short chain fatty acids and "Ivory Soap" into a Thiry-Vella loop.

This generally accepted view was first challenged by Roberts [1931] and Shay, Gershon-Cohen and Fels [1939]. Roberts showed in experiments on man that not only neutral fat, but also free fatty acids, inhibited gastric secretion; Shay, Gershon-Cohen and Fels—also in man—produced marked depression of secretion during and after the intraduodenal instillation of a solution of sodium oleate. Recently Sircus [1958] working with dogs obtained a depression of gastric secretion with the intraduodenal instillation of olive oil incubated with pancreatic juice.

Experiments on dogs [Quigley and Meschan, 1941] and man [Card, 1941] showed that soaps and free fatty acids also depressed gastric motility, and that the products of lipolysis were often more potent inhibitors than the corresponding neutral fat.

Clinical data also point to the greater potency of the products of lipolysis in depressing gastric function. Both in tropical sprue and idiopathic steatorrhœa depression of gastric secretion is a frequent if erratic symptom [v.d. Scheer, 1905; Baumgartner and Smith, 1927; Serra, 1929; Fairley, 1930; Hess Thaysen, 1932; Frazer, 1950] and according to Frazer [1949] decreased gastric motility also is a marked feature of the sprue syndrome. In this syndrome particulate absorption of triglycerides is at fault, and abnormal quantities of fatty acids may occur in the intestine [Frazer, 1950].

The present investigation was undertaken in order to determine the relative potency of neutral fat and certain products of lipolysis in inhibiting gastric secretion in the rat.

METHODS

Operative Procedures.—White male rats weighing 200–300 g. were used. The night before the experiment all solid food was removed and the rats given a 5 per cent glucose solution to drink. In some of the experimental series 2½ per cent casein hydrolysate was added to the glucose solution.

On the day of the experiment a laparotomy was performed under ether anæsthesia, the stomach exposed and a small incision made just proximal to the pylorus. Great care was taken not to injure the blood vessels during this procedure. Two glass cannulæ of 2 mm. internal and 3 mm. external diameter, with a rectangular bend, were introduced through this incision and firmly secured by ligatures. One cannula was introduced caudally through the pylorus into the duodenum, the other pointing orally into the stomach. Both cannulæ carried a length of rubber "drainage" tubing of 1 mm. internal diameter. The tubing on the duodenal cannula was about 45 cm. in length. The distal end was tied, and the whole tubing and cannula filled with the substance under investigation. The tube on the gastric cannula was about 1 cm. long and left open. The purpose of the gastric cannula was to allow the stomach to be emptied at the beginning of the experiment and any food residue to be washed out. The abdominal incision was closed with a continuous suture and the wound painted with celloidin. The tubing of the gastric cannula was then closed with a small artery clip (fig. 1). The rats were put into single wire cages with wire bottoms to prevent coprophagy. The closed end of the duodenal tube was taken through the top of the cage and secured with an artery clip. The rats recovered from the anæsthetic after a few minutes and were able to move about the cage. All injections were given into the duodenal tube without disturbing the rats. At the end of the experiment the rats were sacrificed, the stomach contents aspirated, the volume measured, the pH determined with indicator papers, the free acid titrated with N/100 NaOH, using methyl orange as indicator, and the total chlorides estimated according to Patterson's micro-technique [Harrison, 1943]. In some of the experiments the volume of the small intestine from pylorus to ileocæcal valve was measured and the pH again determined with indicator papers.

In a few of the experiments rats were fitted with a duodenal fistula in order to drain the upper intestinal secretions. A glass cannula of the same design as that used for the stomach was introduced through a small incision in the anti-mesenteric border of the duodenum well distal to the entry of the bile duct and firmly tied in. A glass reservoir of about 3 ml. capacity was attached to the cannula with rubber tubing so that the drained fluid could be collected. A second cannula was introduced immediately distal to the first, through which the substances under investigation could be administered in the usual way.

Method for obtaining a Controlled Fluid Pressure.—A ligature was tied round the duodeno-jejunal flexure. A duodenal cannula was introduced through the pylorus and connected with a glass reservoir of 7 mm. diameter. The whole system was completely filled with the material under investigation, and the top of the reservoir set at the required height above the bottom of the cage. Any increase of intraduodenal pressure was compensated by the spilling of fluid over the top of the reservoir and any reduction of pressure by refilling the reservoir to the original level.

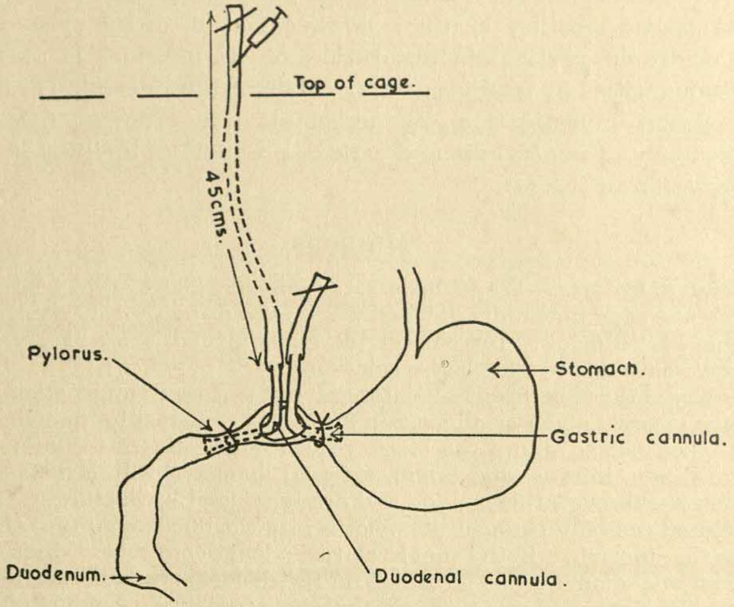

FIG. 1.—Diagram of the operative procedure.

Hydrolysis of Olive Oil.—Olive oil buffered at a pH of 7·8 was shaken at 37° C. with bile salts and pancreatin for 12–24 hr., and the resultant hydrolysate extracted with ether. The ether was evaporated and the residue used.

An analysis of the hydrolyzed olive oil showed the following composition: triglyceride 18 per cent, diglyceride 10 per cent, monoglyceride 10 per cent, free acid 62 per cent.

RESULTS

The Effect of Olive Oil on Spontaneous Gastric Secretion.—Unemulsified undiluted olive oil (BP) was introduced intraduodenally in amounts of 45 mg. every 20 min. over a period of 6 hr. The control animals received the corresponding amount of water. The results are shown in Table I. Under these conditions olive oil did not have any inhibitory effect on gastric secretion. On the contrary, the mean values for both free acid and total chlorides were raised. The difference between the means of controls and tests, however, was not significant at the 0·05 level. The amount of olive oil was therefore increased. Only when 180 mg. were used a significant depression of secretion resulted.

After previous hydrolysis olive oil became a potent inhibitor of gastric secretion. Amounts as small as 26–44 mg. caused a significant depression of free acid as well as total chloride secretion.

TABLE I.—EFFECT OF UNHYDROLYZED AND PREHYDROLYZED OLIVE OIL ON SPONTANEOUS GASTRIC SECRETION

Duration of experiment: 6 hr.

Substance	Amount in mg./20 min.	No. of rats	Mean total chloride in m.equiv.	Mean total free HCl in m.equiv.	S.E.	t	P
Controls (water 0·1–0·2 ml.) .		14	0·84		±0·083	..	..
		18		0·51	±0·066	..	..
Olive oil . .	45	6	1·070		±0·280	1·045	0·4–0·3
		6		0·690	±0·210	1·120	0·3–0·2
	180	8	0·275		±0·099	4·280	< 0·001
		8		0·078	±0·046	4·100	< 0·001
Prehydrolyzed olive oil. .	26–44	5	0·260		±0·071	3·980	< 0·001
		5		0·070	±0·071	3·300	< 0·001

The Effect of Oleic Acid and Sodium Oleate on Spontaneous Gastric Secretion.—As olive oil after hydrolysis had an inhibitory effect, oleic acid, its main constituent fatty acid, was administered under the same conditions. Oleic acid was used both in the free form and as the sodium soap in a 10 per cent solution. The results are shown in Table II. With oleic acid depression of both free acid and chloride was obtained with amounts as small as 10–20 mg., and an increase of the dose to 45 mg. completely suppressed acid secretion. The sodium soap in even smaller amounts (5–10 mg.) depressed secretion and in amounts of 15 mg. or over caused complete suppression.

TABLE II.—EFFECT OF OLEIC ACID AND SODIUM OLEATE ON SPONTANEOUS SECRETION

Duration of experiment: 6 hr.

Substance	Amount in mg./20 min.	No. of rats	Mean total chloride in m.equiv.	Mean total free HCl in m.equiv.	S.E.	t	P
Controls (water 0·1–0·2 ml.) .		14	0·840		±0·083	..	..
		18		0·510	±0·066	..	..
Oleic acid . .	10–20	6	0·470		±0·094	2·450	0·05–0·02
		6		0·280	±0·049	1·930	0·10–0·05
	45	9	0·040		±0·011	7·760	< 0·001
		9		0·000	±0·000	5·370	< 0·001
Sodium oleate .	5–10	7	0·31		±0·087	4·000	< 0·001
		8		0·100	±0·043	3·920	< 0·001
	15–30	5	0·00		±0·000	3·250	< 0·01
		5		0·000	±0·000	4·000	< 0·001

The Effect of Oleic Acid and Sodium Oleate on the Secretory Response to Insulin-induced Hypoglycæmia.—The results are shown in Table III. The amount of insulin used was 0·15 unit/100 g. body weight. With this amount toxic symptoms were avoided and the secretory response of the stomach after 3 hr. was of the same order as the spontaneous secretion after 6 hrs. Both sodium oleate 20 mg. and oleic acid 45 mg., given every 20 min., again produced a significant depression of both chloride and free acid secretion.

TABLE III.—THE EFFECT OF OLEIC ACID AND SODIUM OLEATE ON GASTRIC SECRETION STIMULATED BY INSULIN INDUCED HYPOGLYCÆMIA

Duration of experiment: 3 hr.

Substance	Amount in mg./20 min.	No. of rats	Mean total chloride in m.equiv.	Mean total free HCl in m.equiv.	S.E.	t	P
Controls (water 0·1–0·2 ml.) .		22	0·75		±0·09	..	..
		22		0·53	±0·08	..	..
Oleic acid . .	45	4	0·20		±0·008	2·44	0·05–0·02
		5		0·10	±0·054	2·57	0·02–0·01
Sodium oleate .	20	12	0·075		±0·026	6·34	< 0·001
		13		0·023	±0·012	4·97	< 0·001

The Effect of Draining the Upper Intestinal Juices on the Inhibition of Gastric Secretion by Sodium Oleate.—The same experimental procedures were carried out on rats that had been fitted with duodenal fistulæ in order to study the importance of the upper intestinal juices on the inhibitory mechanism. This time sodium oleate was used and only free acid determined. The results are shown in Table IV. Although the intraduodenal administration of sodium oleate still caused inhibition of gastric secretion, the degree of depression compared with that in the animals without duodenal fistulæ had been significantly reduced (t: 3·1; P: 0·01–0·001).

TABLE IV.—THE EFFECT OF SODIUM OLEATE ON GASTRIC SECRETION STIMULATED BY INSULIN INDUCED HYPOGLYCÆMIA

Duration of experiment: 3 hr.

Substance	Amount in mg./20 min.	No. of rats	Type of preparation	Mean total free HCl in m.equiv.	S.E.	t	P
Controls (water) .	20–50	14	Duodenal fistula	0·596	±0·089	..	..
Sodium oleate .	20	19	Duodenal fistula	0·302	±0·076	2·5	0·02–0·01

The Effect on Gastric Secretion of Distending the Small Intestine with Liquid Paraffin.—In order to assess what effect distension of the small intestine alone might have on gastric secretion, liquid paraffin was introduced intraduodenally in a single amount of 5 ml. and a ligature tied immediately beyond the material administered. The effect was studied on spontaneous secretion (fig. 2) as well as on insulin stimulated secretion (Table V). Spontaneous secretion was completely suppressed, and the response to insulin induced hypoglycæmia significantly reduced.

When the site of administration was changed to the middle of the jejunum, or to the more distal portion of the ileum, no inhibition of gastric secretion resulted (Table V).

Comparison of the Inhibitory Effect of Olive Oil on Gastric Secretion Stimulated by Insulin-induced Hypoglycæmia with that of Liquid Paraffin.—In this series of experiments olive oil and liquid paraffin were administered intraduodenally in equal amounts. The rats were fitted with duodenal fistulæ to avoid the effect of the additional volume of upper intestinal juices. The amount of oil was 270 mg. every 20 min. for 3 hr. and insulin-induced hypoglycæmia was again used as the stimulus for gastric secretion. The results with olive oil were erratic. Complete or almost complete depression of free acid secretion occurred with three of the six rats examined. With liquid paraffin depression of secretion occurred in four of the six rats used and there was no significant difference between the means (t: 0·32). Doubling the amount of liquid paraffin did not enhance its inhibitory effect.

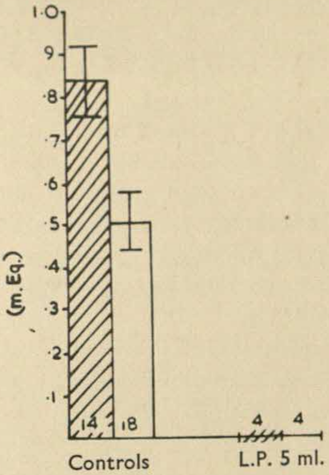

Fig. 2.—The effect of the intraduodenal administration of a single dose of liquid paraffin on spontaneous gastric secretion in conscious rats. Pylorus and duodenum ligated. Duration of experiment 6 hr. The hatched columns represent the means of total chlorides and the clear columns the means of the free acid secreted. The bars represent the standard errors of the means. The numbers refer to the numbers of animals used. L.P. = liquid paraffin.

Table V.—The Effect of Distension with a Single Dose of 5 ml. of Liquid Paraffin on Gastric Secretion Stimulated by Insulin Induced Hypoglycæmia.

Duration of experiment: 3 *hr.*

Site of distension	No. of rats	Mean total chloride in m.equiv.	Mean total free HCl in m.equiv.	S.E.	t	P
Controls (no distension) .	22	0·75		±0.09	. .	. .
	22		0.53	±0·08	. .	. .
Duodenum . . .	7	0.12		±0·05	4·56	< 0·001
	7		0·06	±0·04	3·33	< 0·001
Jejunum or ileum . .	7	0·55		±0·09	1·14	0·3–0·2
	7		0·44	±0·10	0·73	0·5–0·3

The Effect of Pressure Changes in the Duodenum on the Inhibitory Effect of Distension on Gastric Secretion. (*Comparison of the Effect of Sodium Oleate with that of Water.*) In these experiments a ligature was tied around the duodeno-jejunal flexure, the duodenum was distended with sodium oleate and the distending pressure kept constant throughout the experimental period of 3 hr. Insulin-induced hypoglycæmia was used as the stimulus for gastric secretion. Fig. 3*a* shows a significant correlation between the distending pressure and the inhibition of gastric secretion (r: −0·622; P: 0·01–0·001). When water was used instead of sodium oleate (fig. 3*b*) a significant correlation (r: −0·516; P: 0·05–0·02) was again found, but water was less potent in depressing gastric secretion than sodium oleate.

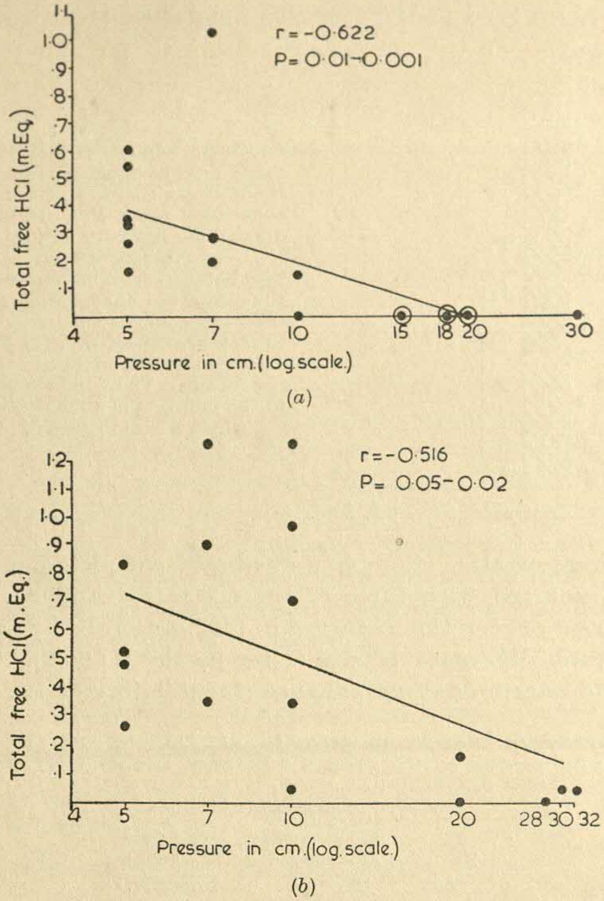

Fig. 3.—The effect of intraduodenal pressure on gastric secretion of free HCl by conscious rats. The pressure is expressed as the height of the fluid level above the wire bottom of the cage (log. scale). r: correlation coefficient. The lines are calculated regression lines.

(*a*) the effect of 10 per cent sodium oleate.

(*b*) the effect of water.

Discussion

Piontkowsky [1904] and Babkin [1905], and recently Sircus [1958] administered fatty acid intraduodenally into dogs and found a stimulation of gastric secretion. The reason for the difference in the two species is not clear. Unfortunately, neither the concentrations of the solutions nor of the exact position of the fistulæ are stated by the earlier workers. A possible stimulating effect of weaker concentrations would be more easily detected in dogs, who frequently have no fasting gastric secretion [Babkin, 1950; Necheles, 1955] than in rats, who secrete large amounts of acid gastric juice when fasting.

Since the experiments of the Pavlov school [Szokolov, 1904 and Lönnquist, 1906] it has been accepted that the inhibitory effect of fat on gastric secretion is due to its presence in the intestine. During the present investigation it was found that inhibition of gastric secretion was frequently associated with the accumulation of large amounts of fluid in the intestine at the end of the experimental period. This suggests that the upper intestinal juices might play a part in the inhibitory mechanism. Draining the upper intestinal juices significantly reduced, but did not abolish, the inhibitory effect of sodium oleate. Under these conditions the volume of the intestinal fluid found at the end of the experiment was reduced, but was still larger than that of the control animals. These findings suggest that distension of the intestine with fluid might be an important factor in the inhibitory mechanism of fat on gastric secretion.

The inhibitory effect of distension of the upper small intestine on gastric secretion in dogs was first mentioned by Day and Webster [1935] and later shown to vary with the degree of the distending pressure [Sircus, 1953; Walker, 1955]. It is well known that hypertonic solutions of glucose are potent inhibitors of gastric secretion in man and dog [Muir, 1949], and it seems likely that the superiority of intraduodenal hypertonic glucose over that of intravenous glucose as an inhibitor of gastric secretion [Muir, 1949] is due to the duodenal distension it evokes.

In an attempt to demonstrate the effect of distension on gastric secretion, unemulsified liquid paraffin was introduced intraduodenally in the present investigation and prevented from passing down the intestine by a ligature just beyond the material administered. A significant inhibition of both spontaneous secretion and insulin stimulated secretion was found. With changing the site of administration to the jejunum or the ileum, no depression of gastric secretion occurred. These findings suggested that the inhibitory mechanism operated from the duodenum only and not from the more distal portions of the small intestine. If the distending material was allowed to pass down the intestine, depression of gastric secretion still occurred, but the results became erratic. There was no difference between the inhibiting effect of olive oil and liquid paraffin under these conditions. The fact that increasing the volume of the single dose of distending material from 0·3 ml. to 0·6 ml. did not increase the effect indicated that the distending pressure in the duodenum was a more important factor in this mechanism than the volume of fluid administered. This was confirmed by the finding of a negative correlation between distending pressure in the duodenum and the degree of free acid secretion by the stomach. Such a negative correlation was found with water, as well as with sodium oleate. Sodium oleate, however, seemed to inhibit gastric secretion more than water at the same distending pressure.

In the sprue syndrome several factors contribute to cause distension of the small intestine. Absorption is generally depressed and increased amounts of free fatty acids may be present in the intestine. Further small intestinal motility is reduced and there is excess mucus secretion; also an increase of volatile fatty acids, due to fermentation, is a frequent occurrence. The radiological picture of the distended small intestine after the administration of non-flocculating barium is one of the characteristic features of the syndrome.

The present findings in the rat suggest that such distension of the duodenum might be a major factor in producing hypochlorhydria or achlorhydria as they frequently occur in the sprue syndrome, and might also account for the erratic nature and the reversibility of these symptoms.

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THE EFFECT OF HYPERVITAMINOSIS A ON BLOOD COAGULATION IN THE RAT. By J. C. F. POOLE.* From the Sir William Dunn School of Pathology, Oxford.

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Rats fed vitamin A in doses of 40,000 i.u. daily develop a coagulation defect after about 10 days. The same result is produced by synthetic vitamin A as by naturally occurring vitamin A in a fish-oil concentrate. The thrombin generation test is a more sensitive indicator of the coagulation defect than either the calcium time or the one-stage prothrombin time.

The development of this defect can be prevented by the simultaneous administration of vitamin K_1 or menaphthone provided that treatment is continued for long enough. The nature of the defect, appears, however, to be different from that induced by dicoumarol poisoning.

The appearance of defective coagulation is accompanied by a striking localization of sudanophil material in the liver in the Kupffer cells: this change is not prevented by vitamin K_1 or menaphthone. Several other manifestations of hypervitaminosis A are also unaffected by vitamin K_1 or menaphthone.

HARRIS and Moore [1928] showed that overdosage with vitamin A led, among other things, to a hæmorrhagic condition. Rodahl and Moore [1943] demonstrated that the hæmorrhagic illness in man caused by eating polar bear or seal liver could be accounted for by the very high concentrations of vitamin A which they found in the livers of these animals; they produced a hæmorrhagic disease in rats by feeding polar bear liver. Light, Alscher and Frey [1944] measured the one-stage prothrombin time [Quick, 1935; Campbell *et al.*, 1941] in rats fed vitamin A in excess and showed that it was prolonged; the prolongation could be prevented by giving vitamin K_1 (2-methyl-3-phytyl-1: 4-naphthoquinone) at the same time. The findings of Light *et al.* were confirmed and extended by Walker, Eylenberg and Moore [1947] who found that menaphthone (2-methyl-1: 4-naphthoquinone) acted similarly to vitamin K_1 in preventing the development of a prolonged one-stage prothrombin time after excessive vitamin A feeding. These findings suggested that the concentration of some clotting factor might be controlled by the action of two of the fat-soluble vitamins. In view of current interest in the effects of fat feeding on blood coagulation, a re-investigation of the effects on coagulation of hypervitaminosis A seemed desirable.

METHODS

Animals.—Experiments were carried out on Wistar strain albino rats, of either sex, bred in this laboratory. In all, 104 animals were used. Weights ranged from 200–350 g.

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* Locke Research Fellow of the Royal Society.

Diet and Administration of Vitamin Preparations.—The rats were given a stock diet and water *ad libitum* during the course of the experiments. Vitamin A was given as liquor vitaminæ A concentratus B.P., which is a fish-oil concentrate and contains approximately 40,000 i.u./ml. in a vegetable oil, or as synthetic vitamin A palmitate dissolved in olive oil in equivalent concentration. The rats receiving vitamin A were given 1 ml. of one of these preparations daily by stomach tube under light ether anæsthesia. This method allows the natural route of absorption with precise control of the dose. Control animals were given 1 ml. olive oil daily in the same way. Vitamin K_1 (Mephyton, Merck) and Menaphthone (Synkavit, Roche) were diluted with normal saline to give 65 μg. and 25 μg. respectively in 0·2 ml., which amount was given daily by subcutaneous injection.

Collection of Blood.—Blood was obtained by cannulation of the aorta using the method of French [1955] and mixed with 3·8 per cent trisodium citrate solution in the proportion of 9 parts of blood to 1 part of citrate solution. In each case the first 4·5 ml. of blood obtained was run directly into the citrate. Plasma was collected after centrifuging the citrated blood at 4000 r.p.m. for 20 min.

Clotting Tests.—(1) Calcium time. A mixture of 0·1 ml. plasma + 0·1 ml. 0·85 per cent sodium chloride solution was recalcified with 0·1 ml. of 0·025 M calcium chloride solution and the clotting time was recorded. (2) One-stage prothrombin time. A mixture of 0·1 ml. plasma + 0·1 ml. brain extract was recalcified with 0·1 ml. 0·025 M calcium chloride solution and the clotting time was recorded. The brain extract was prepared as follows. The brains of thirty normal rats were freed from meninges and vessels and ground up in acetone with a pestle and mortar. The residue was collected in a Buchner filter and the acetone extraction repeated three times. It was then dried on filter paper at 37° C. in an incubator and stored at room temperature; 200 mg. of this residue was extracted with 4 ml. of 0·85 per cent sodium chloride solution at 37° C.; the saline suspension was allowed to stand for 30 min. and the opalescent supernatant liquid pipetted off and used for the tests. (3) Thrombin generation test [based on the methods of Macfarlane and Biggs, 1953; and Pitney and Dacie, 1953]. A mixture of 0·5 ml. plasma + 0·5 ml. 0·85 per cent sodium chloride solution was recalcified with 0·5 ml. 0·025 M calcium chloride solution. At 1-min. intervals up to 10 min. after adding the calcium chloride, 0·1 ml. of this mixture was added to 0·4 ml. of a 1 in 1000 solution of fibrinogen. The clotting times of the fibrinogen samples were determined: graphs were drawn in which the time after recalcification was plotted against the reciprocal of the fibrinogen clotting time (which is approximately proportional to the concentration of thrombin). The thrombin generation test was the principal test used in this investigation and it was necessary to find some way of reducing the mass of data collected to manageable proportions. Inspection of the results suggested that the area enclosed by the curve was the most important parameter of the thrombin generation curves as far as changes revealed in this study are concerned. Therefore most of the results of thrombin generation tests are reported as a quantity which it is proposed to call the cumulative thrombin production, this being defined as $\sum_{t=0}^{t=10} \frac{1}{F}\delta t$, where F is the fibrinogen clotting time in seconds and t is the time after recalcification in minutes. (4) Two-stage prothrombin time. A mixture of 0·5 ml. plasma + 0·5 ml. brain extract (prepared as described above) was recalcified with 0·5 ml. 0·025 M calcium chloride solution. At 1-min. intervals up to 6 min. after adding the calcium chloride solution, 0·1 ml. samples of this mixture were added to 0·4 ml. of 1 in 1000 fibrinogen solution, and the clotting times of the fibrinogen samples were determined.

Histological Preparations.—Sections of liver, spleen and retrosternal lymph nodes were examined. The material was fixed in formalin and frozen sections were stained by Harris' hæmatoxylin and Herxheimer's Sudan IV.

RESULTS

The Effects of Hypervitaminosis A on the Calcium Time, the One-stage Prothrombin Time and the Thrombin Generation Test

The results of typical clotting tests on two pairs of rats are shown in fig. 1. In each case, a rat which had received vitamin A was compared with one which had received olive oil for the same period. The duration of treatment was from 11–14 days. It is seen that all three tests demonstrated a coagulation defect in the rats given excess vitamin A, but that the thrombin generation test was the most sensitive indication of abnormality. Indeed, when the extreme ranges of the values obtained in the thrombin generation test for five pairs of control and test animals are plotted on the same graph (fig. 2) there is practically no overlap. For most of the remaining animals used in this study, only the thrombin generation test will be reported.

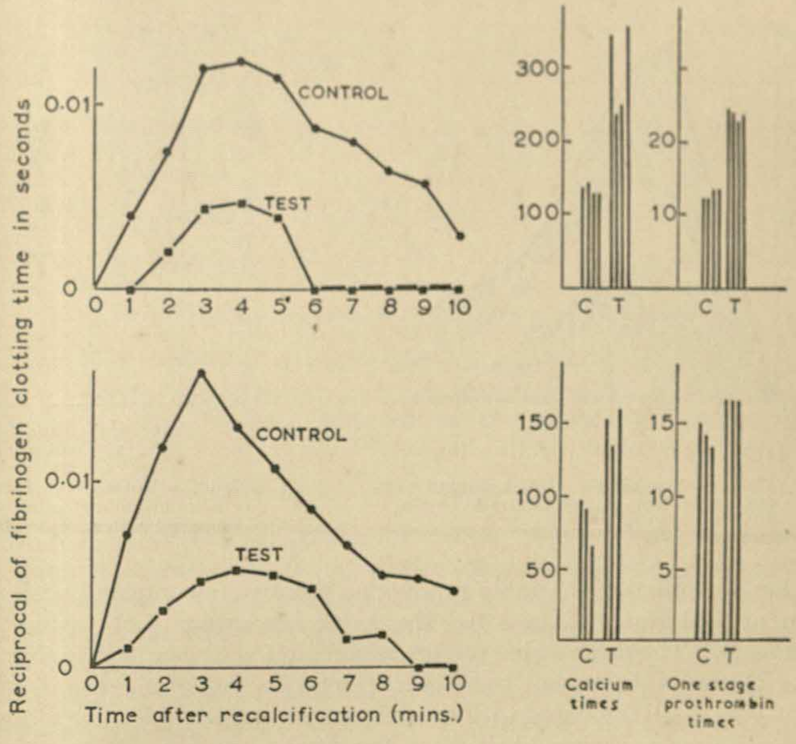

FIG. 1.—Thrombin generation tests, calcium times and one-stage prothrombin times for two pairs of rats. In each, the control rat (C) had received 1 ml. olive oil daily and the test rat (T) 1 ml. liquor vitaminæ A conc. B.P. daily. Three or four replicate determinations of the calcium time and one-stage prothrombin time are recorded for each animal, the columns representing individual determinations.

The Effect of Olive Oil Feeding on the Thrombin Generation Test

Control animals used in this investigation were given 1 ml. of olive oil daily. This was done because the vitamin A preparation used was dissolved in a vegetable oil and was given in doses of 1 ml. daily. To justify this control procedure it was necessary to examine what effects, if any, such olive oil feeding has on the thrombin generation test. The cumulative thrombin production in rats fed olive oil for periods of from 1 to 31 days showed no tendency to increase or decrease significantly during this period of time. In fig. 3 these cumulative thrombin production figures are compared with those for ten normal rats (*i.e.* rats fed the stock laboratory diet without any supplement). The mean value for the rats fed oil is 21 per cent higher than that for the normal rats, but there is considerable overlap and the difference is in any case small compared with the difference demonstrable after vitamin A feeding. Indeed it seems likely that the results would have been closely similar if normal rats had been used as control animals.

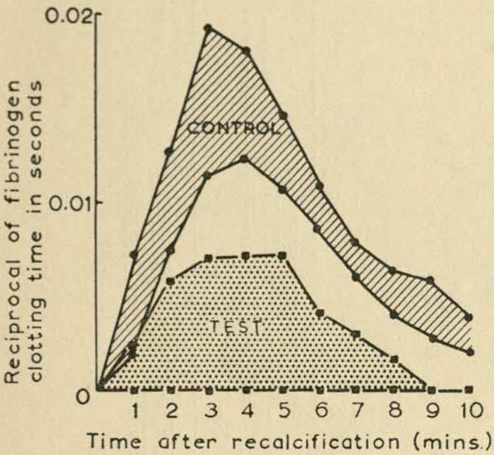

FIG. 2.—The extreme ranges of the results of thrombin generation tests carried out on five pairs of rats.

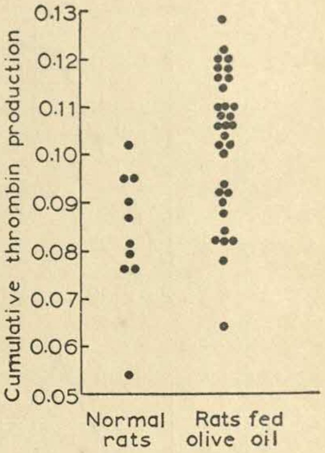

FIG. 3.—Cumulative thrombin production values for normal rats compared with rats fed olive oil.

The Extent of the Clotting Defect produced by Vitamin A in relation to Duration of Treatment

Fig. 4 shows cumulative thrombin production figures for thirty-two rats given vitamin A (as liquor vitaminæ A concentratus B.P.) and killed at periods ranging from 1 to 31 days after starting treatment. All the rats which had had vitamin A for more than 10 days showed substantially defective thrombin production and so did one rat treated for 7 days, but the other seven rats which had been treated for less than 10 days showed figures within the normal range (*cf.* fig. 3). Each rat treated with vitamin A was killed at the same time as a control rat fed olive oil for the same period, so it is possible to express the same results in a different way, namely, as percentages of the control value. This has been done but the elimination of certain technical errors which can be achieved in this way did not significantly reduce the overall variability of the results. It seems likely, therefore, that there is a considerable variation in the response of individual animals to the same dosage of vitamin A. Because of this variation, it is impossible to decide, on the basis of these results, whether or not there is a further progressive change in coagulability of the blood after the defect has become established.

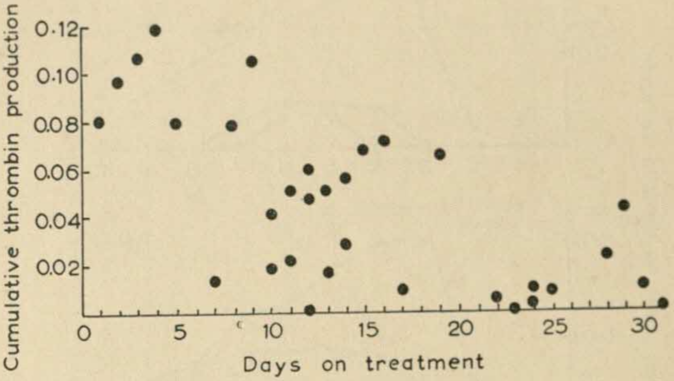

FIG. 4.—Cumulative thrombin production values for rats fed 1 ml. liquor vitaminæ A conc. B.P. daily for varying periods.

The Effect of Synthetic Vitamin A Palmitate

Fig. 5 shows thrombin generation curves obtained with the plasma of three rats which had been fed synthetic vitamin A palmitate compared, in each case, with a rat fed olive oil and a rat fed liquor vitaminæ A concentratus B.P. for the same length of time. The two vitamin A preparations, which were administered in equimolar amounts gave closely similar results. These findings would seem to remove any doubt as to whether the activity of the fish-oil concentrate is due to vitamin A or to some other substance.

The Effect of Vitamin K_1 and Menaphthone in opposing the Anticoagulant Effect of Vitamin A

The results of the present investigation are in general agreement with those of Light *et al.* and Walker *et al.* in that it was found that both vitamin K_1 and menaphthone are capable of abolishing the anticoagulant effect of vitamin A under certain circumstances. This statement must be made, however, with the reservation that in three rats menaphthone apparently did not have this effect; these animals had been treated for 10, 13 and 16 days respectively. No such case occurred among animals given vitamin K_1 but the numbers involved are not sufficiently large to warrant the conclusion that the action of vitamin K_1 differs in this respect from that of menaphthone.

Examples of cases where menaphthone did and did not abolish the effect of vitamin A are shown in fig. 6. The complete set of results with menaphthone and vitamin K_1 are expressed as cumulative thrombin production values in figs. 7 and 8.

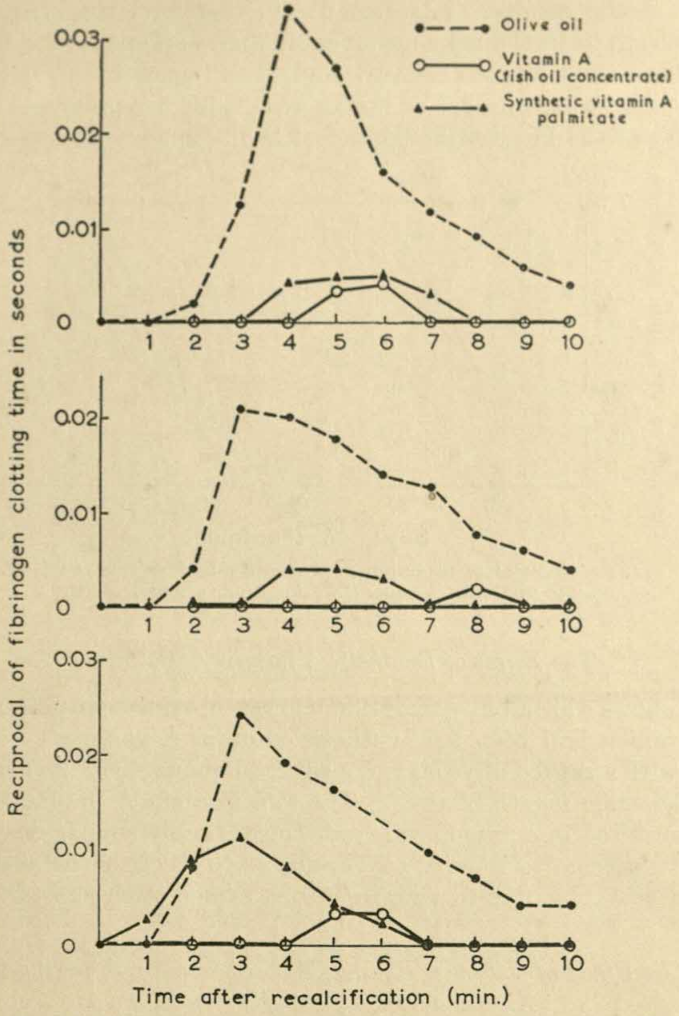

FIG. 5.—Thrombin generation tests on three sets of three rats. In each set, one rat had received 1 ml. olive oil daily, one had received 1 ml. liquor vitaminæ A conc. B.P. daily, and one had received 1 ml. synthetic vitamin A palmitate in olive oil daily.

Correlation of Clotting Deficiency with Changes in the Histological Pattern of Fat Distribution in the Liver

Two strikingly different patterns of incorporation of fat in the liver were encountered. The animals fed olive oil, and also the animals fed vitamin A which showed no clotting defect, had sudanophil material in most of the

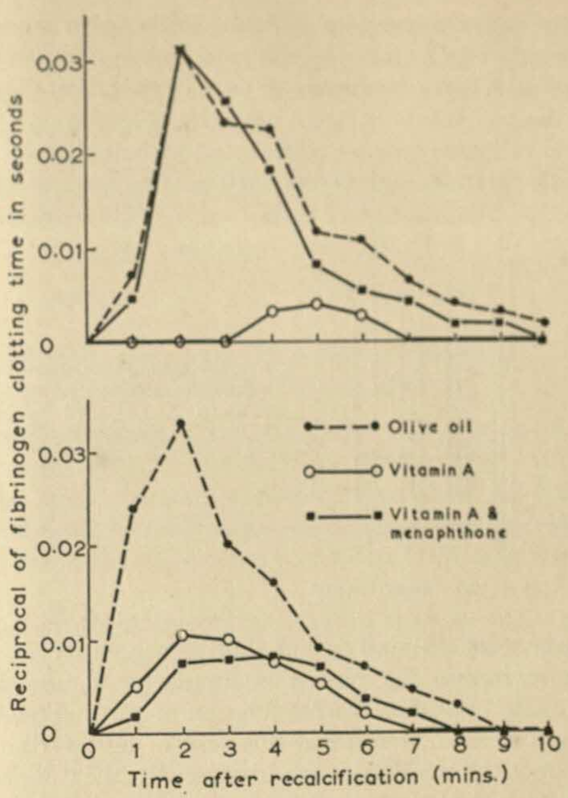

FIG. 6.—Thrombin generation tests showing the varying effects of simultaneous administration of vitamin A and menaphthone.

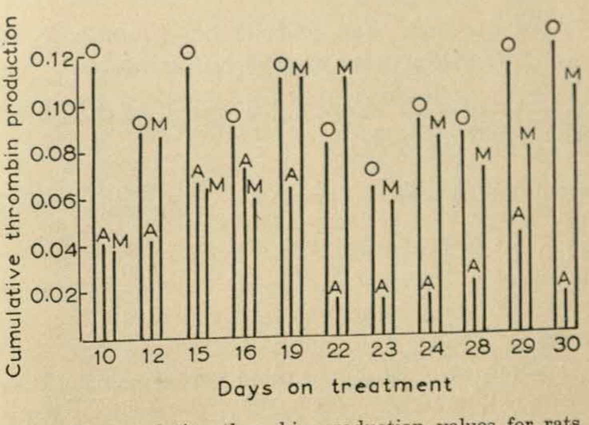

FIG. 7.—Cumulative thrombin production values for rats receiving olive oil (O), vitamin A (A) and vitamin A with menaphthone (M).

liver cells arranged in a random fashion. The other animals all showed marked concentration of sudanophil material in the Kupffer cells as has been observed before in hypervitaminosis A [see Popper, 1944]. In the animals fed vitamin A the correlation between defective coagulation and accumulation of fat in Kupffer cells was striking: all the animals with defective coagulation showed this pattern; none did whose clotting tests gave values in the normal range. However, the animals which had received both vitamin A and menaphthone or vitamin K_1 showed accumulation fat in the Kupffer cells whether they exhibited defective coagulation or not. Some of the animals which were given vitamin A and had this type of fat distribution in the liver also showed sudanophil material in macrophages in the lymph nodes. Where this occurred not all the lymph nodes examined were involved. It is possible that if more lymph nodes had been examined the phenomenon would have been observed more often. In no case did the spleen show any significant deposition of sudanophil material.

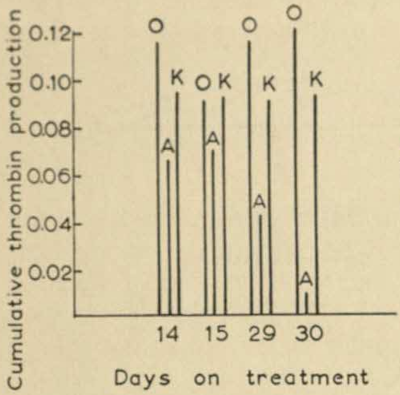

FIG. 8.—Cumulative thrombin production values for rats receiving olive oil (O) vitamin A (A) and vitamin A with vitamin K_1 (K).

The Relationship of Menaphthone and Vitamin K_1 to other Manifestations of Hypervitaminosis A

The animals treated with vitamin A showed marked loss of weight as is illustrated in the following table:

Treatment	Olive oil	Vitamin A	Vitamin A + menaphthone	Vitamin A + Vitamin K_1
Weights (g.) of 4 rats in each group.	370	290	285	170
	380	230	260	220
	300	230	250	240
	300	360	250	270

Period of treatment: 4 weeks.
All rats weighed between 300 and 350 g. at the beginning of the experiment.

It is clear that menaphthone and vitamin K_1 do not prevent loss of weight. Many of the rats which had been given vitamin A for 3 weeks or more showed a tendency for the hair to fall out. The hair could easily be removed by plucking. Loss of hair in rats with hypervitaminosis A was observed by Harris and Moore [1928]. The simultaneous administration of vitamin K_1 or menaphthone did not prevent this occurrence. Most animals given excess vitamin A for more than 10 days showed a greater lipæmia than did control animals given the same volume of olive oil for the same period. A moderate depression of the packed cell volume was also often noticed. These conditions were not affected by simultaneous administration of vitamin K_1 or menaphthone.

Further Observations on the Nature of the Coagulation Defect produced by Hypervitaminosis A

In cases where the plasma showed a prolonged calcium time, mixing it with an equal volume of normal plasma produced a normal calcium time. This indicates that the abnormality is brought about by a deficiency of some clotting factor and not by the presence of a circulating anticoagulant. The prolonged calcium time is not restored to normal by adding normal serum to the plasma.

The two-stage prothrombin time showed changes analogous to those revealed in the thrombin generation test. A representative example of the findings with the two-stage prothrombin time in a hypervitaminotic and a control rat is shown in the following table:

Minutes after recalcification	1	2	3	4	5	6
Fibrinogen clotting times in seconds for test carried out with:						
(*a*) Control rat . . .	23	71	540	>600	>600	>600
(*b*) Rat fed excess vitamin A	364	223	590	>600	>600	>600

DISCUSSION

At the time when Light *et al.* and Walker *et al.* carried out their earlier studies on the coagulation defect in hypervitaminosis A in the rat, it was generally believed that prolongation of the one-stage prothrombin time indicated prothrombin deficiency. This view is no longer tenable for reasons which were fully discussed by Biggs [1953]. The belief that both excessive feeding with vitamin A and the administration of dicoumarol (and similar naphthoquinone derivatives) caused prothrombin deficiency led naturally to the successful attempt to correct the deficiency in hypervitaminosis A with vitamin K_1 and its synthetic analogue menaphthone. In the light of subsequent knowledge the apparent antagonism between vitamin A and substances with vitamin K activity is puzzling. Dicoumarol and its analogues are generally believed to act by competing with vitamin K, their similar chemical structure suggesting that they might be antimetabolites of vitamin K [Hunter and Shepherd, 1955; Albert, 1956]. But the chemical structure of vitamin A shows no obvious similarity to that of the active naphthoquinone derivatives. In any case, the results of the present study would seem to rule out the possibility of a direct competition between vitamin A and vitamin K inasmuch as all the other manifestations of hypervitaminosis A which were observed were unaffected by substances having vitamin K activity. Moreover it appears that the nature of the coagulation defect in hypervitaminosis A is different from that in dicoumarol poisoning; in the latter case the defect in plasma coagulation can be corrected by adding normal serum. Indeed, the coagulation defect in hypervitaminosis A appears to be a genuine prothrombin deficiency in so far as this can at present be defined [see Biggs and Douglas, 1953].

The findings in those animals treated with both vitamin A and menaphthone were unexpected and may be of wider interest. There have been many reports of the ineffectiveness of menaphthone treatment in experimental and clinical situations where it might have been expected on theoretical grounds to correct a clotting defect [see Mann, Mann and Bollmann, 1950, for a full discussion]. The possibility that such treatment might have been effective if it had been continued longer does not seem to have been considered. The present results probably indicate that there is a considerable delay period before menaphthone treatment becomes effective in abolishing the anticoagulant action of vitamin A.

The correlation between defective coagulation and accumulation of fat in the Kupffer cells of the liver is striking: the coagulation defect correlates much better with this histological change than it does with the duration of vitamin A administration. It is difficult to believe, however, that there is a direct causal connexion, as vitamin K_1 and menaphthone do not influence the histological appearances.

The fact that vitamin A has an anticoagulant effect must raise the question whether it could be of value for therapeutic purposes in man. It would have several obvious advantages: it is cheap, readily available and can be given by mouth; an antidote is available. Nevertheless it would seem that there are serious toxic effects of vitamin A which accompany its anticoagulant action. Unless these changes are readily reversible, or do not occur in man, it is unlikely that vitamin A has any future as a therapeutic measure in thrombotic conditions.

ACKNOWLEDGMENTS

I thank Professor Sir Howard Florey, F.R.S. and Dr. R. G. Macfarlane, F.R.S., for constant advice and encouragement; Mr. H. W. Wheal, Mr. P. Harvey and Mr. D. W. Jerrome for technical assistance; and Dr. H. Harris who read the manuscript and made valuable suggestions.

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BOOK REVIEWS

SELECTED SCIENTIFIC PAPERS FROM INSTITUTO SUPERIORE DI SANITA, ROME. Editor E. B. Chain. Oxford: Blackwell Scientific Publications Ltd. 1957. Vol. 1, part 3, pp. 216. 57s. 6d.

This important volume contains a review of present knowledge of the mechanism of the mode of action of insulin and sets out a new theory.

Claude Bernard's account of the production of artificial diabetes by wounding the floor of the fourth ventricle was published in 1849. Bernard made use of Fehling's method for the detection of sugar in the urine which had been published the previous year. From then for about a 100 years, it has been taught that diabetes is primarily a disease of carbohydrate metabolism. This conception really arose as an accident from the ease with which sugar could be detected and measured in urine and blood. Modern methods of biochemistry have shown beyond doubt that diabetes is a general metabolic disorder affecting carbohydrate, fat and protein metabolism. This, as Chain and his colleagues point out, is what makes diabetes so complex a subejct for study.

All metabolic reactions which are stimulated by insulin are of a synthetic nature and the insulin effect is an immediate one. Insulin accelerates glycogen synthesis, fat synthesis, protein synthesis and growth. All these synthetic reactions have in common the property that they require a considerable energy supply and to carry them out the energetic potential of the cell must be raised. This the authors postulate is precisely what insulin is doing.

How insulin might bring about these syntheses is discussed. The action is rapid and could not be brought about by a simple increase in enzyme substrate concentrations. The energy potential of the cell is characterized by the facility with which the cell can accumulate energy rich bonds. The synthesis of these bonds in animal tissues is an ærobic oxidative process, ultimately linked with the different redox systems of the respiratory chain. The review ends with a discussion of the possibility that the hormone exerts its function by raising the energy potential of the cell by releasing the reduced form of a redox system such as DPN : DPNH from a protein bound form. The reduced form is then available for the formation of energy rich phosphate bonds.

For both physiologists and physicians the mode of action of insulin is a key biochemical problem. This volume can be strongly recommended as a clear presentation of a modern view.

R. PASSMORE.

PROGRESS IN BIOPHYSICS AND BIOPHYSICAL CHEMISTRY. Vol. 8.

Edited by J. A. V. BUTLER and B. KATZ. London: Pergamon Press, Ltd. 1957. Pp. viii + 409. £5, 5s.

A marked change in emphasis has occurred in this series since the first volume was published in 1950. In that the emphasis was firmly placed on the biophysical techniques. Gradually, however, the proportion of articles reviewing the biological significance of results obtained with such techniques has increased. This trend, reflecting as it does the increasing use of physical methods by biologists in general, must have added considerably to the value of this series to an ever-widening circle of readers. The change of emphasis is so marked in this, the eighth volume of the series that the most logical way of reviewing the individual contributions is to group them according to the type of biological problems discussed.

There are two articles on special senses, each excellent in its own way, but presenting a marked contrast in approach. That by Whitfield on hearing covers the whole subject, beginning with the reception of sound waves at the cochlea and ending with the arrival of auditory information at the cortex. That by Brindley on colour vision is restricted to a discussion of events in the most peripheral part of the visual pathway. Though some sections are aimed at the specialized reader, the overall result is a clear, logical account of human colour vision.

The three longest articles in this volume all deal with cell membranes. The first, by James, is a full account of the application of electrochemical measurements to studies of the bacterial surface. Both the technicalities of this type of measurement and the implications of results so far obtained are adequately discussed. The second, by Schmitt and Geschwind, is an admirable review of our present knowledge about the nature of the axon surface. They deal largely with structural problems and say little about the permeability properties of the nerve membrane. They do, however, consider in some detail the immediate environment of the axon surface, both inside and outside, and the bearing it may have upon the subject of synaptic transmission. In the third of these articles, Glynn discusses at some length the ionic permeability of the red cell membrane. This contribution is notable for the clear account of the many and varied flux measurements which have been made on red cells and of the interesting conclusions which can be drawn from them.

Desoxyribosenucleic acid is the subject of two articles differing greatly in scope. The longer article, by Shooter, is an excellent review of the enormous help which accurate physico-chemical measurements are making to our understanding of this complex molecule. The other, by Kelly, is largely an account of experimental studies on the effects of radiation on mammalian cells and is, perhaps, too narrow in conception to be wholly suitable for inclusion in this series.

The remaining two articles have a more biochemical flavour and deal with the biosynthesis of complex macro-molecules. In the first, concerned with the formation of connective tissue, Smith attempts to cover a very amorphous field, not yet ripe for a review of this sort. In the second, Loftfield gives a clear picture of recent biochemical evidence for the mechanism of protein synthesis and, if the last few pages are highly speculative, they form a fitting end to an excellent volume.

This volume is the longest of the series, and also much the most expensive. If these two facts are directly related it is perhaps unfortunate that one or two of the shorter articles were not omitted since the high price may discourage many research workers from buying it for their own book-shelves, a place where some of the longer articles deserve to be. There are a few misprints, mostly typographical in nature and not scientifically misleading. There is no strict uniformity in the presentation of references: one would like to see all the references given with titles plus first *and* last pages. These are but minor criticisms however, for judged as a whole the volume is a valuable addition to this excellent series.

P. R. Lewis.

THE PHYSIOLOGY OF FISHES. Vol. 2. BEHAVIOUR. Edited by Margaret E. Brown. New York: Academic Press Inc. 1957. Pp xi + 526 with 93 illustrations and 17 tables. £5.

To a mammalian physiologist the strongest impression that results from a reading of this excellent book is that information on the mechanisms of fish behaviour is both scanty and disjointed. It must be added that this impression is not the fault of the several authors concerned but rather of the width of the subject and the small amount of work that has so far been done on it.

Each of the fourteen chapters reviews a different subject and is by a different author. The first, by Healey, discusses the nervous system. This is rather compressed, but gives a good survey of experiments dealing with the effects of lesions of various parts of the nervous system on behaviour: while much conflicting information has been gathered on rhythmic swimming movements, it is remarkable how little is known about the functions of the different parts of the brain or about the electrical activities of the nervous system; this particular field is wide open for further study. Brett, in his article on the eye, gives a brief (too brief) account of ocular anatomy and physiology, together with descriptions of some odd functional adaptations. It would be of interest, for instance, to know something of the central organization of the visual pathways of the "four-eyed fish", *Anableps anableps*. The chapter on water quality requirements by Doudoroff tends, unavoidably, to be a miscellany; it is also rather disconcerting to come across the words "acclimatization to heat", and "heat-acclimated" on the same line (p. 407). The article on physiological genetics by Gordon is good and would have been better if a brief explanation of some of the technical terms had been given. The remaining articles in this book are competently written while that by Keynes on electric organs and Lowenstein's review of the acoustico-lateralis system are excellent.

The reference lists are extensive and there are good author and subject indexes. The volume is well produced and well illustrated; it makes exciting reading and will probably have the effect of sending physiologists off hot-foot to the marine biological establishments in an attempt to fill in the gaps.

R. M. Gaze.

THE LIPIDS, THEIR CHEMISTRY AND BIOCHEMISTRY. Vol. 3. BIOSYNTHESIS, OXIDATION AND METABOLISM. By Harry J. Deuel, Jr. New York: Interscience Publishers. 1957. Pp. xxxvi + 1065. $25.00 (188s.).

Professor Deuel died before this monumental volume was published. The magnitude of the task which he undertook during his last years is apparent in the size of the book; despite his illness the layout, style and presentation of the subject matter is up to the high standards set in the two previous volumes. This volume contains extensive reviews of the biosynthesis, oxidation and metabolism of all the common lipids by the animal body, a dissertation on the question of the conversion of fat to carbohydrate, the metabolism of branched chain acids, cholesterol, vitamins A, D, E and K, the physiology of essential fatty acids, and concludes with an extensive review of the nutritional value of fats.

Advances in lipid metabolism will require modification of some of the detail in this volume but for years to come when "pre-1956 data" on lipids is required most investigators will automatically go to "Deuel". The admiration, gratitude and indebtedness of many to Professor Deuel will accumulate throughout the years for this outstanding contribution to an important branch of the biological sciences.

G. S. Boyd.

THE CEREBROSPINAL FLUID. A Ciba Foundation Symposium. Edited for the Ciba Foundation by G. E. W. Wolstenholme and Cecilia M. O'Connor. London: Churchill. 1958. Pp. xii + 335. 50s.

By penning up, for a few days, a number of active contributors to a subject on which much work is being done and causing them to think and talk, the Ciba Foundation provides something infinitely more valuable than the conventional textbook or review. This book is readable. It contains fifteen separate contributions, followed by frank discussion: the level of presentation is simple and fairly free of specialized jargon. The print and illustrations are excellent.

The contributions dealing with mainly structural aspects seem to show no revolutionary disagreement with earlier ideas, but in certain matters it is clear that controversy exists. Turner, for instance, doubts the existence of a true sub-arachnoid space in the parasagittal region of primates, the fluid presumably percolating through a fibrous meshwork there. Woollam and Miller find no capillaries in the subarachnoid space, and therefore doubt such theories as propose the formation or absorption of C.S.F. in this space. They also look on the pericapillary and perineuronal spaces in the central nervous system as artefacts, so that only ground substance and glial feet come between the capillary wall and the nerve cell. Miss Cooper describes nerve fibres, though no encapsulated endings, accompanying all blood-vessels of the choroid plexus: it is not known if these are motor or sensory. Kappers describes phases in the embryonic development of the human telencephalic choroid plexus from a hæmopoietic tissue, through a period in which it contains a gelatinous stroma full of glycogen, to a more mature villous form; and he considers that the plexus reaches structural maturity only after birth. It is relevant here that in another article Herlin, studying the increased penetration of bilirubin from blood into brain in the presence of the SH inhibitor p-chloromercuribenzoate, again raises the question of immaturity of functional "barriers" and points out that bilirubin penetrates more readily in immature animals (kernicterus). This question has obvious pathological significance.

Three other papers on the choroid plexus admit tentative theories of action there. Ladman and the late George B. Wislocki, describing appearances in electron micrographs, indicate a possible cycle of structural changes in the epithelial cells, which show much variation in apical distension; and this might have secretory significance. Lumsden, in examining the villous cysts which form in tissue cultures of choroid plexus, finds dye concentration by the cysts and also an "apocrine" extrusion of globular balls of material from the cells. Bowsher shows a higher sodium concentration in fluid taken from the exposed plexus than in free ventricular or subarachnoid fluid; though the technique is not claimed to have great accuracy.

Four papers deal with the interchange of water and electrolytes between blood, C.S.F. and brain. Selverstone shows that ^{24}Na exchanges more quickly with ventricular fluid than with the subarachnoid space, and that the exchange of ^{42}K is more rapid near the choroid plexus than elsewhere in the lateral ventricle; but he shows a commendable reluctance to draw any conclusions from these data as to secretion. Less directly, he shows that the uptake of ^{24}Na into the ventricular C.S.F. is more rapid than its uptake in the opposite direction. Davson considers the nature of the C.S.F. and its relationship to extracellular fluid and the neurone; and he raises a question of extreme interest to nerve physiologists—whether the extracellular fluid is a simple filtrate of plasma or whether, being derived by some secretory process, it has a different composition. He shows that it is unlikely to be a filtrate, because the chloride of C.S.F. is too homogeneous to admit its being subject to free diffusion with a fluid so different from itself as a plasma filtrate. Further, inhibition by Diamox slows the penetration of ^{24}Na from the blood into C.S.F. but not into cerebral extracellular fluid; so the formation of extracellular fluid may be a separate active process. This brings to the fore, not for the first time, the idea that central nerve cells and fibres may be exposed to a fluid very different in ionic composition from the plasma filtrate in which one imaginatively places them.

As elsewhere in physiology, many of those working in this field appear to be preoccupied with the immense difficulties in interpreting experiments carried out by new methods such as electron microscopy or the use of radioactive isotopes. In a discussion of the relation of modern work to the classical studies by Weed, Selverstone comments sagely that "a rather unphysiological experiment which is correctly interpreted may be more valuable than a highly physiological [isotope] experiment which is misinterpreted". The contributors to this symposium show a reasonable amount of mutual caution which is survived by some interesting ideas.

G. C. Gordon.

AN INTRODUCTION TO EXPERIMENTAL SURGICAL STUDIES. By W. J. Dempster, F.R.C.S., Lecturer in Experimental Surgery, Postgraduate Medical School of London. Oxford: Blackwell Scientific Publications. Pp. 463. 50s.

In his introduction the author states that the task of the experimental sugeon involves more than operative interference, and that his function is to discover the causes and conditions which make surgical interference a necessity. The more obvious aim of the experimentalist is to develop satisfactory techniques with the help of animals other than man. To satisfy both these aims, the experimental surgeon must perforce interest himself in physiology, biochemistry, biophysics and so on, not only as they apply to man but also in their comparative aspects. Thus the author is stressing that all biology is one and that any specialist is simply a man who looks at a large field with numerous objects from a given point of view: few have time to wander round and appreciate it from all angles. It is, therefore, helpful to have a number of varied and relevant facts collected and sorted. The book is arranged in sections dealing with aspects of surgery such as tissue regeneration and tissue grafting and implanting, and with different parts of the body such as the cardiovascular system, the thorax, the genito-urinary organs and the digestive tract. Two chapters are given to low temperature studies and hypertensive diseases. In each chapter there is a description of the normal reactions and control of the tissues or organs, our knowledge of the factors causing disease and how equivalent pathological states can be produced and alleviated experimentally. In addition, mention is made of the possible surgical approaches to a problem and the pros and cons of the methods used by different people.

The author has succeeded in packing a great deal of information into his book, and as well as the facts and references, offers criticism and asks questions. This stimulates the reader to think for himself. One virtue of the book is the attention it draws to the many outstanding unsolved problems of surgery and physiology (using the latter term in the widest sense) and it should help both clinical and academic workers to see how much they need each other.

There are gaps in the book, the most evident being the lack of any reference to work on the central nervous system. This aspect of surgery is advancing rapidly and its importance to the patient is immense in that it may directly affect personality. There are, too, the many problems relating to the effect of surgery on the peripheral nervous system. But this is a specialized subject and it is too much to expect one man to cover all aspects of experimental surgery. The book is readable and may be recommended to anyone, surgical or non-surgical, who cares to learn how his subject appears from the viewpoint of an experimental surgeon.

Mary Pickford.

BOOK NOTICES

Causation of Cancer. Edited by E. Boyland. *Brit. med. Bull.* **14**, Number 2. 1958. Pp. 196. 25s. This timely symposium was produced in anticipation of the Seventh International Cancer Congress in London in July 1958. Twenty-seven well-known British workers contribute to a balanced and scholarly review of the whole subject. There has been much growth of knowledge of the nature of chemical carcinogens since a previous review on this topic was published (*Brit. med. Bull.* **4**, 1947). Other fields in which there has been progress include: tumour immunology, immunogenetics, endocrine and radiation carcinogenesis and the study of vital statistics. This number of the British Medical Bulletin comes up to the high standard set by previous issues and can be confidently recommended as an up-to-date, well documented and authoritative review of a complex and expanding field of knowledge. The Introduction provides a pleasant tribute to the life and work of the late Sir Ernest Kennaway.

Die Fruhdiagnox in der Luchtung und Zuchtungforschung. Edited by H. Stubhen and W. Schmit. Berlin: Springer. 1957. Pp. 96. D.M. 20. In selecting plants or animals (for economically important characters) progress is often considerably hindered by the length of the generation-interval or the rarity of the most desirable types. In such cases it is sometimes possible to accelerate matters by selecting on the basis of some features of the juvenile organisms. The theoretical and practical possibilities of such methods have been discussed by a number of authors in a recent special number of Der Zuchter, with special, but not exclusive, reference to the breeding of trees.

Animal Behaviour. Vol. 6. Numbers 1–2. London: Baillière Tindall & Cox Ltd. 1958. The British Journal of Animal Behaviour has changed its name, consequent upon the addition of American colleagues to the editorial staff. The editors hope to be able to publish a greater flow of material on topics related to animal behaviour. The first number under the new title is well printed. The text figures are clear and the photographic plates excellent. Some physiologists may find this journal very suitable for the publication of their results. Many of the papers in this issue are of a general interest and should appeal to all biologists.

Connective Tissue. Edited by R. E. Tunbridge, M. Keech, J. F. Delafresnaye and G. C. Wood. Oxford: Blackwell Scientific Publications. 1957. Pp. xii + 371. £2, 2s. This is a record of a symposium organized by The Council for International Organizations of Medical Sciences. The meetings were held in London in July 1956, and were attended by scientists from all countries, including U.S.S.R. and Hungary. Modern sophisticated biochemistry techniques for separation and analysis are being increasingly applied to the various proteins and polysaccharides of connective tissue fibres; the majority of the papers at this symposium were concerned with the physico-chemical nature of the extracellular material characteristic of these tissues. Work is, however, reported which attempts to elucidate the cellular formation of collagen. Some apparently opposing viewpoints were resolved into mere differences of nomenclature and definition and this may perhaps have been the most valuable contribution of this conference.

Water and Electrolyte Metabolism in Relation to Age and Sex. Ciba Foundation Colloquia on Ageing. Vol. 4. Edited by G. E. W. Wolstenholme, O.B.E. and M. O'Connor. London: J. &. A. Churchill Ltd. 1958. Pp. xi + 327. £2, 5s. This is one of a series of colloquia on the biological problems of ageing; it will, however, be of interest to a much wider range of physiologists and clinicians than those specifically concerned with old age. For, quite rightly, as much attention was given to the functional behaviours of the infant kidney as to that of the senile individual. Moreover, fundamental aspects of the cellular and extracellular control of electrolytes and water were also discussed and the interpretation of the "brief" was wide enough to include work on electrolytes and congestive cardiac failure.

HANDBUCH DER MIKROSKOPISCHEN ANATOMIE DES MENSCHEN. By W. v. Möllendorff. Fortgeführt von W. Bargmann. Bd. IV, Nervensystem. Teil 4: Das Kleinhirn. By J. Jansen and A. Brodal. 1958. Pp. 323, 197 illustrations. D.M. 142. Recent advances in our knowledge of the anatomy of the cerebellum, in great measure, are the result of the work of the Oslo School and in particular of the authors of this excellent monograph. It is small wonder, therefore, that this addition to the encyclopædic *Handbuch* gives an authoritative account of the morphology, histology, fibre connexions and blood supply of the cerebellum and brings together a mass of recent information particularly on the connexions between the brain stem and cerebellum. Physiological considerations are not neglected and the illustrations, bibliography and production are excellent.

SHERRINGTON: PHYSIOLOGIST, PHILOSOPHER AND POET. By the Rt. Hon. Lord Cohen of Birkenhead. Liverpool University Press. 1958. Pp. xiv + 108. 17s. 6d. This contains an accurate account of the main events in Sherrington's life, a painstaking and indeed pedestrian account of his contributions to physiology and a patronizing chapter on his philosophy. The chapter on Sherrington's verse does not make one want to read the original poems.

THE EXCITABLE CORTEX IN CONSCIOUS MAN. By Wilder Penfield. Liverpool University Press. 1958. Pp. ix + 42. 10s. 6d. This is a brief account of some selected experiences of the author and his patients and an interpretation in terms of cortex and centrencephalic system. It is a pity that one who has access to such fascinating material should make so few logical inferences from it.

ESSENTIAL FATTY ACIDS

Editor: H. M. SINCLAIR

Some 30 years ago it was shown that certain unsaturated fatty acids are needed in the diet of rats and therefore constitute vitamins. In the last year or two it has been found that these acids lower serum cholesterol and that deficiency of them may be relevant to certain very important clinical conditions such as atheroma and coronary thrombosis.

Our understanding of the structure and synthesis of fatty acids has increased enormously in recent years. Their chemical, biochemical and clinical aspects were thoroughly discussed at the Fourth International Conference on Biochemical Problems of Lipids of which this volume is a record. Its forty-two edited papers adds considerably to current knowledge of this rapidly advancing subject. **Price 50s.**

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